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Abstracts of 14th FELASA congress 2019

OA1S1

How do we currently view teaching a ''Culture of Care''? Introduction & Feedback

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Abstract
Nurturing a ''culture of care'' and impacting professional attitudes in the field of animal research remains challenging due to its social and ethical implications, and the need for long term engagement of all the trainees. From an educational perspective, current practice remains challenged by the variable inter-cultural perceptions on animal welfare, uncertainty on the harmonisation of training programs between institutions, the limited reflective teaching approaches and valuable expert formative assessment of care and welfare and the lack of strategies to ensure the long term professional engagement on care and welfare. Unfortunately many existing training programmes on animal experimentation are focussed on technical training, highlighting an urgent need for better constructive alignments approaches to genuinely make and impact the professional commitment towards culture of care in staff and students working in animal research.

During this introduction feedback acquired during a previous successful London meeting (2018) held on the topic will be disseminated. The organisers will share their passion for this topic and the scene will be set for a series of guest speakers to share their thoughts and expertise on this subject.

The specific objectives of the session are:

1. To share good practice, resources and potential solutions that delegates could implement into their own research activities in order to improve education in a culture of care
2. To promote collaborative working and networking between researchers, animal technologists, and animal welfare organisations
3. To foster increased collaborations between FELASA and its membership with reflective teaching and educational practices in this area

OA1S2

Research Culture, Learning Trends and Education in animal studies

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Abstract
One of the biggest factors that will influence how an individual conducts their research is the research culture that they work within. Often within the laboratory animal sciences this is also referred to as the local '*culture of care*'. Unfortunately the phrase '*culture of care*' will mean different things to different people. For some individuals '*culture of care*' is about caring for the subjects of the research such as the experimental animals by implementing the 3Rs and improving animal welfare. For others it is about caring for the beneficiaries of the research, such as patients, by ensuring the research conducted is rigorous and reproducible. '*Culture of care*' can also relate to the help and support provided by the research establishment or employer through education, training and mentoring or by those in management positions. Thus the first step towards achieving a good '*culture of care*' is to define it at a local level. Once defined it becomes possible to design education, training and mentoring provision to support its delivery. This presentation will discuss '*culture*' before focusing upon key learning trends and how to utilise them to deliver timely and engaging education and training that supports professional development and delivers culture change.

OA1S3

Engaging staff in animal welfare

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Abstract
Key to the work of animal technologists is their ability to care about (the suffering of, the quality of life of) as well as care for (in terms of husbandry) the animals they work with. This capacity to care about is a quality actively sought when research facilities recruit staff, but it is also something hard to define, hard to teach and difficult to measure. It is key, we are told, to what makes a good animal technologist, and consequently to both staff and animal welfare. Yet is also something placed at risk by a role where workers are continuously exposed to procedures and

protocols which cause (albeit within strictly regulated legal limits) harm, suffering and death to the animals they care for. Drawing on over 5 years of social science research with staff in UK animal facilities, and in particular animal technologists, this paper explores the key role played by care and emotion in ensuring good animal welfare, how this produces a particular division of emotional labour within research facilities and how we might better support and learn from animal technologists as part of building a culture of care.

OA1S4

Educating animal welfare

Arndt Saskia
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Abstract
Educating animal welfare is of high importance within animal experimentation from both, an ethical point of view and for supporting the generation of reliable, replicable and valid experimental results. Attitude and critical reflection on own ways of thinking and acting are essential when working with laboratory animals. These considerations are the corner stones of our education philosophy and -practice at Utrecht University. Since 1986, when we offered the first course on laboratory animal science worldwide, we gained a lot of knowledge regarding educating animal welfare. During this presentation I will share our insights and best practices when dealing with course participants of different personal, professional and cultural backgrounds within animal welfare related teaching activities. Special attention will be given to our approach, in which we focus within welfare education not only on the scientific but also on the ethical, emotional and cultural aspects.

OA2S1

The FELASA accreditation scheme according to the Directive 2010/63/EU: 2014–2019

Gyger Marcel, Berdoy M., Dontas I., van Ginneken C., Kolf-Clauw M., Linklater N., Steidle J. and Sjöquist M.
FELASA Accreditation Board for Education and Training

Abstract
Since 1995, date of its first publication on education of people involved in animal experimentation, FELASA has been on the forefront on education and training in Laboratory Animal Science in Europe. Following enforcement of the Directive 2010/63/EU, the FELASA Accreditation Board for Education and Training, http://www.felasa.eu/accreditation-board-for-education-training, has adapted its 2002 recommendations several times on the FELASA website before publishing a revised open access paper. The scheme will be briefly presented from application to annual reporting and accreditation renewal.

A key feature of the FELASA accreditation scheme is auditing courses immediately after accreditation has been granted. The audit process includes management of the course as well as its educational dimension. Focus is on how practical sessions are carried out and how the students' new acquired skills are evaluated. Interviewing relevant stakeholders such teachers, participants of the past and current course, researchers and principal investigators having themselves attended such course or sent their researchers to the courses, allows the board to give to the course organizer advice and commendations, in addition to recommendations. A summary of the key findings of audits will be reported.

The Directive and the European Commission guidance document has put emphasis on the Learning Outcomes (LOs) of the different modules. Using a core module as an example, a survey on how LOs are evaluated by the course organisers of FELASA accredited courses will be presented.

OA2S2

The GV-SOLAS accreditation scheme of Laboratory Animal Science Courses

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Abstract
To standardize the curricula of LAS-courses, the GV-SOLAS (Gesellschaft für Versuchstierkunde, Society of Laboratory Animal Science) has been accrediting LAS-courses in Germany and Austria since the late 1990s, according to the accreditation scheme based on the FELASA recommendations for people either conducting animal experiments (FELASA Cat B) or being responsible for planning procedures (FELASA Cat C). With the Directive 2010/63/EU and with the corresponding changes in the German Legislation including the ''Tierschutzversuchstierverordnung'' being slightly different from the European regulations, the GV-SOLAS adapted its accreditation scheme to fit the new legal requirements. The new scheme is comprised of a modular, species specific set-up based on the recommendations by the EU-Expert Working Group for Education and Training. Course organizers apply to GV-SOLAS by providing information on course infrastructure, lecturers and tutors, ethical approval of working with live animals as well as complete course documentation. The GV-SOLAS Education and Training board evaluates courses by reviewing all course materials, including each lecture and any supplementary materials. A focus is laid on current, animal welfare conform methods and the application of the 3Rs. Accreditation is granted for the period of three years after which a course can be re-accredited. As part of the accreditation scheme, course organizers are requested to have their course(s) evaluated and to provide a summary of the evaluation and possible actions taken to the board as part of the reaccreditation process. Further, GV-SOLAS provides a sample certificate that takes German as well as EU requirements into account.

OA2S3

The UK Accreditation Scheme

Berdoy Manuel
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Abstract
Since 1st April 1994, the UK Competent Authority (Home Office) has required all personnel carrying out animal experiments (what is now defined as Function A) to have successfully completed an accredited training programme before a licence can be applied for under the UK Law (Amended 2012, to transpose European Directive 2010/63/EU). From 1995 this also became a requirement for persons responsible for directing animal experiments (Function B).

Course Accreditation is carried out by three Accrediting Bodies recognised by the Competent Authority: The Royal Society of Biology, the Scottish Accreditation Board and the Universities' Accreditation Group. Courses are accredited for five years following successful application and a visit to the institutions. Renewal of Accreditation follows a similar process. Since 2014/15 the Accrediting Bodies have overseen the update of course content to reflect the modular structure and Learning Outcomes detailed in the EU Education and Training Framework and endorsed by the UK Competent Authority.

I will present a short overview of the relevant main features of current UK Accreditation System and the issues that arise from it.

OA2S4

Something old, something new and something blue – FELASA accreditation in aquatic organisms

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Abstract
EU Directive 2010/63/EU demands professional and species-specific expertise of experimenters working with laboratory animals. Therefore, the long-existing and well-recognized FELASA courses were adapted according to the new regulatory requirements. These courses mainly focussed on the use of mammals, especially rodents. The increasing use of zebrafish in research required addition of zebrafish modules to the course portfolio to offer appropriate training. Especially, there is still a very wide gap in laboratory animal work between mammalian and aquatic species with only very few overlap in personnel. The European Zebrafish Resource Centre (EZRC) built up a completely new training course focusing mainly on zebrafish and Medaka to fulfil the needs of the experimenters and the requirements of legislation. While parts of the core modules such as legislation and ethics can be adopted from other courses, aquatic organisms have some peculiarities to consider: (1) procedures to be applied are very different. Surgeries are much more uncommon while *in vivo* imaging is much more prominent. In case of surgeries, these are very specific treatments often only occurring in specialized labs. (2) Most research projects are performed in larval or embryonic stages that do not require an authorization and are not regulated by legislation. (3) Welfare assessment in fish is much behind that of rodents and diagnostic options are limited. (4) Animal handling mostly bases on tank recognition and not on individual animals. We are presenting our ideas in setting up and running a course considering the differences and still fulfilling regulatory and scientific needs.

OA2S5

Role of AFLAS and its constituent associations in education and training in Asia region

Ingle Arvind
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Abstract
Providing Training and Education is an integral part of any scientific association. Internationally goal of humane care and use programs are '*Animal Welfare*'. In Asia region, AFLAS has been supporting various member countries undertaking education and training course in laboratory animal science through ''Education and Training Activities for Laboratory Animal Science and Technology'' (ETALAST). This program was established at the AFLAS Council meeting held in Singapore in 2015. So far eight member countries have received financial support from AFLAS under this program which I will detail.

Laboratory Animal Scientists Association (LASA) India is a national scientific association dedicated to advancing laboratory animal science. LASA India is 'Member' of AFLAS Associations since 2011. LASA has been undertaking the task of conducting training and education for the laboratory animal professionals. LASA India has a policy of providing funding support for the members to hold the regional workshop/ training program/ conferences. LASA India has also articulated with the TANUVAS to start the first FELASA course outside the Europe in 2013.

ACTREC is premier Autonomous institute under Department of Atomic Energy, Govt. of India undertaking basic research in all aspects of cancer. ACTREC is an Institutional member of ICLAS since 2004. ACTREC has been regularly conducting the training and workshops in laboratory animal science since 2005. With our efforts ICLAS has recognised us one of the centres for ''Training and Education'' in this field. ICLAS has even permitted us to use their logo for certificates issued to the participants of training conducted by us.

OA2S6

Sustainable laboratory animal sciences education and training for researchers; Lessons from the Emerging World

Lewis David
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Abstract
Laboratory animal sciences is a global endeavour, essential to the understanding of biology, and the development of new medicines. Legislative, ethical review and the education and training requirements for laboratory animal science personal differ substantially across the World. Many countries in the Emerging World are using the development of their pharmaceutical industries as a route to national economic growth. However, like countries in the Developed World, they suffer from a shortage of suitably trained laboratory animal sciences personnel.

To address this shortage, the International Union of Basic and Clinical Pharmacology established its IOSP initiative, its objective, to work with colleagues in the host countries or regions, to co-design and co-deliver introductory education and training in laboratory animal sciences for early- career researchers. This initiative has expanded massively over the last 8 years. It now provides education and training, and continuing professional development activities for undergraduates, both early career and established researchers, vets and para-vets, animal technologists and facility managers. To ensure sustainability, it has moved towards ''Train the Educator/Trainer'' courses, where participants use the educational resources, and the knowledge and skills gained from these courses to deliver similar courses in their own Institutions and networks. Its activities to date have focused in Africa[1], China[2] and India[3], but it would welcome potential collaborators in other parts of the world.

This initiative demonstrates how, by working collaboratively with colleagues across the world, we can share good practice in laboratory animal sciences globally, to the benefit of animals, science and humanity.

OA3S1

Why quality assurance of veterinary training is so important?

Iatridou Despoina
Federation of Veterinarians of Europe (FVE), Brussels, Belgium

Abstract
The primary responsibility for education and vocational training in EU belongs to the Member States (MS). EU is only mandated to carry-out actions to support, coordinate or supplement the actions of MS.

Since long, FVE advocates for quality assurance of education and training. High quality training is a precondition for reliable qualifications that can be recognised in other countries.

FVE has taken important initiatives to support implementation of quality assurance of veterinary training.

Regarding graduate level, FVE together with EAEVE (Academia) have established the European System of Evaluation of Veterinary Training (ESEVT) to ensure that graduate-veterinarians acquire the technical skills, knowledge and attitudes necessary to start in a variety of roles in veterinary medicine (Day-One-Competences). Veterinary profession is proud that ESEVT is the first profession-specific European quality system that is recognised and accredited by ENQA [''Top level Quality Assurance for Education in Veterinary Medicine in Europe (2018)''].

Regarding post graduate training, FVE calls on all veterinarians, irrespective of their areas(s) and level of activity, to remain up-to-date through continuing education, in line with EU recommendations and the FVE Code of Conduct. FVE highlights that quality assurance of postgraduate training is necessary to facilitate recognition of competences acquired after graduation by the veterinary licensing bodies [''FVE position on Continuous Professional Development (2018)'', "OIE recommendations (2016)"]. This is why FVE together with EAEVE and EBVS (Veterinary Specialists) have jointly established VETCEE-Veterinary Continuous Education in Europe to promote the above principles and facilitate recognition of postgraduate competences acquired through CPD.

OA3S2

How do you become an ECLAM Diplomate?

Hedenqvist Patricia, Kalman R., Whelan G., Seebeck P., Zeiter S., Ritskes-Hoitinga M., Hardy P. and Gilbert C.
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Abstract
The need for specialization in veterinary medicine is growing. In laboratory animal medicine (LAM), post-graduate training is a driving force to improve research quality and animal welfare. The fact that ECLAM training includes a research component promotes the development of LAM, increases the credibility of the specialist in the research community and facilitates collaboration. ECLAM strongly encourages authorities and animal research organizations to support specialist education. ECLAM is also a member of IACLAM, connecting to colleagues in Asia and US. Diplomate status is the highest level of veterinary specialisation.

ECLAM training is available via standard or alternate-route programs. Prerequisites are graduation from an EAEVE-accredited veterinary school and one year of internship. Both routes require three years of training under the supervision of an ECLAM Diplomate and 60–100% of time dedicated to LAM. An equally qualified non-Diplomate can also serve as a local supervisor in the alternate program. Requirements on elements of training, animal species and facilities are detailed on the ECLAM website. Two scientific publications (one of them as first author) are required.

After completion of training, the resident submits his/her credentials application to the College for evaluation. Credentialed candidates are eligible to take the 3 paper written exam, which takes place once a year. If successful, the candidates continue to the oral/practical exam. In the last three years, 17 residents have taken the exam and 10 are now among the 71 certified European Specialists in Laboratory Animal Medicine.

0A3S3

Institute for Laboratory Animal Management – a unique learning experience!

Helppi Jussi
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Abstract
Directors, managers, and supervisors of laboratory animal facilities have seen their roles grow more and more complex over the years, and they have found themselves in a unique profession. People in management positions must be able to interpret the social, political and economic environments in which they operate and use administrative and technical skills to maintain and improve their animal care and use programs. The vast majority of persons in these positions were trained in biologic and veterinary sciences and must find additional training in management skills to fulfill their administrative roles. The Institute of Laboratory Animal Management (ILAM) is an AALAS educational program founded in 1992. It is developed to provide instruction in management concepts that are applicable to the communication, team building, and networking among colleagues with mutual interests. The program is a fully immersive, intensive management and leadership training program over a 2-year period, and it is held at a facility in Memphis, USA that is specifically designed for educational programs, meetings, and conferences. Class topics vary from year to year depending on the needs of the industry and the requests of the students, including i.e. Facility Technology, Problem Solving, Team Building, Performance Appraisals and Financial Management. ILAM is an excellent opportunity for all people in management level positions to expand their knowledge of management skills, as well as being an exceptional change for networking with other professionals on the field.

0A3S4

Education, Training and Continuing Professional Development Activities in Laboratory Animal Science in South Africa

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Abstract
Recent years have witnessed a major renaissance in laboratory animal science (LAS) and ethics in South Africa and Africa, with substantial growth in education and training (E&T) and continuing professional development (CPD).

A watershed was the 2015 conference of the South African Association for Laboratory Animal Science (SAALAS), establishing that national E&T standards should harmonise with international best practice. E&T and competence assessment were key themes in the 2017 SAALAS-ICLAS (International Council for Laboratory Animal Science) international conference in Cape Town, attended by 143 delegates from 23 (including 7 African) countries.

SAALAS has since established an annual national training day for Animal Ethics Committees (AECs); a 5-day train-the-trainer workshop in 2018 for veterinary and para-veterinary professionals in best practice in LAS (collaboration with Nordic Consortium for Laboratory Animal Science, Leeds University and University of Zürich); and is initiating the training of the first laboratory animal technologists in 20 years. Online training for AECs is a current priority.

The University of Cape Town – as a hub of E&T for the African continent – has developed a 40-hour training course based on the principles of FELASA-accreditation, attended by 30 institutions, aimed at persons who conduct procedures, care for and euthanase animals; and is developing courses for animal caretakers, facility managers and animal technologists.

As member of the ICLAS Africa Regional Commi ttee, SAALAS works closely with the Pan-African Network in Laboratory Animal Science and Ethics (established 2017 at SAALAS-ICLAS conference), to ensure sustainable E&T programmes in Africa.

0A4S1

Exploring Active Learning: from flipped classroom to virtual reality

Laurent Xavier, Berdoy M. and Ramadan T.
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Abstract
The education literature suggests that students must do more than just listen: they should read, write, discuss, create content or be engaged in solving problems with peers (Steen-Utheim & Foldnes, 2017). Most importantly students must engage in higher-order thinking tasks (high level of cognitive activity) such as analysis, synthesis, and evaluation (Bonwell & Eison, 1991).

As part of its new Digital Education Strategy Oxford University is trying to promote active learning more widely and this talk will address the following two points:

1) show an example of the use of a flipped classroom' approach to address some of the leaning outcomes of Module 20 (Anaesthesia for minor procedures) in the FELASA accredited function A course at Oxford University using the dedicated emodule resource
2) show examples of digital teaching practices and innovative technologies can be put in practice in teaching students.

OA4S2

Laboratory Animal Science online training through the eyes of the learner: perceptions of gains

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[3]*FPCEUP, Faculty of Psychology and Education Sciences of the University of Porto, Porto, Portugal*

Abstract
The European directive 2010/63/EU issued clear recommendations concerning the training and competence of researchers using animals in experimental procedures. Different courses and training programs have emerged across Europe, and the Laboratory Animal Science community is facing the challenge of offering qualified and flexible training. From similar educational areas, E-learning appears to be a promising solution tool, fulfilling the flexible-time training and quality criteria.

However, very few studies have addressed the participants' perception of e-learning as a training tool. For course organizers the e-learning approach is the key to the problem of demanding programs and short training periods. However, participants' perceptions, that is known to be a relevant factor for learning have not been investigated.

Within an exploratory study framework, the e-learning approach perception of 229 researchers participating in LAS training programs was assessed. Participants answered the Questionnaire of E-learning Acceptance (QELA), a Likert-type scale comprising 5 subscales that explores how participant perceive the organization of the e-learning, its relevance for the time management and for preparing for practical training. Results also explored the influence of student's sociodemographic and professional profile in the e-learning acceptance.

In general, e-learning was students' well accepted by the majority of the participants as an alternative to classroom lectures. We will further discuss how we have used the results to improve our use of e-learning.

OA4S3

How can E-learning resources contribute to meeting the training requirements of EU2010/63?

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Abstract
EU Directive 2010/63 requires appropriate training of various individuals with responsibilities for the conduct of studies that involve the use of laboratory animals. Delivery of training to this large number of individuals (several 1000 in the UK each year) is already resource intensive, and the required time and effort of trainers is likely to increase. Provision of E-learning modules to support this training can help with meeting the requirements of the Directive in a cost-effective way.

We launched three E-learning modules (EU5, 6 and 20) between 2014 and 2016 and the first of a series of modules related to EU21 was recently launched with support from the UK NC3Rs. The modules are developed using Articulate E-learning software and are delivered both as standalone modules (https://www.nc3rs.org.uk/3rs-resources) and via Learning Management Systems (LMS) enabling progress tracking for course leaders (www.flairelearning.com and www.researchanimnaltraining). Since launch, over 6000 individuals have completed one or more modules, and they have been used as part of a blended learning approach by the developers in Newcastle, Stockholm and Coimbra. Other training providers in the UK and in other member states have also incorporated the material into their courses. User feedback has been excellent, and it has enabled reduction of the face-to-face component of these taught courses, so reducing the trainer-resource required.

We are currently developing E-learning modules for project applicants, project assessors and ethical review committee members, with funding from the European Commission. These latter modules will be freely available via a platform managed by ETPLAS.

OA4S4

Distance learning in vocational education and training – collaboration between Sweden and South Africa

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Abstract
''The best week ever that I will remember for a long time. How is it possible that I have learned so much in one week?'' /Course participant

Online learning platforms with flexible settings for users and content is an efficient way of outreach between educators, learners and professionals. The needs of different learner categories and professional requirements can be satisfied and adjusted concomitantly, both in terms of challenges and contents. The platform continues living as an everyday reference working tool and secure international communication channel amongst professionals.

This scenario is implemented in a collaborative initiative between South Africa and Sweden via the Nordic Consortium for Laboratory Animal Science. A one-week hands-on workshop for

veterinarians, veterinary nurses, technicians and animal caretakers was integrated with, and preceded by, exposure to online training content. Online engagement significantly reduced traditional lecture time, which was replaced by case studies, discussions, debate and practical sessions. Attendees had a broad range of professional and socio-economic backgrounds, as often seen in lower- and middle-income countries, approaching learning from diverse experiential viewpoints. Participants were never allocated to groups based on professional or other hierarchies.

Participant feedback overwhelmingly reflected modern evidence-based theory of optimised learning and knowledge retention. The strongest positive themes reported by participants included a sense of equality, calmness and confidence (no fear); appreciation for vocational case-based learning; transparency in learning goals, methods and contents; few traditional lecture methods; and methods of assessment consisting of evidence-based competence standards, instead of traditional marks, grades or other norm-referenced criteria.

0A5W1

How to put the fun back into learning?

Kerton Angela
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Abstract
Are you interested in game–based learning? Then come play games with us during our FELASA workshop! Whether you teach senior professors or technical staff, you will learn some fundamentals of educational game design that will improve engagement and retention with your staff and students.

This workshop is designed to assist trainers who want to start using interactive challenges to help them educate staff in laboratory animal science, whether this is an integral part of compulsory training courses, ''refresher'' type training or self-development by increased knowledge through Continued Professional Development (CPD).

Included in this workshop is a presentation focussing on setting up interactive educational practices within the laboratory animal technology field, exploring possible solutions to improving better education and engagement through Game Based Learning (GBL). Suggestions of how GBL can be applied within the laboratory animal science field and guidance on how to ensure success for their implementation will also be discussed. There will opportunity for delegates to try out examples of GBL tool(s) in groups or teams e.g. scratch cards quizzes, missing words / task challenges or an interactive audience ''game'' such as ''Blockbusters''

The specific objectives of the session are:

1. To provide examples of more participative and interactive approaches such as interactive challenges and game-based approaches to support motivation, learning, and change
2. To demonstrate benefits and efficacy of new game-based teaching approaches in the laboratory animal science field and share new interactive educational skills that delegates can integrate into their own workplace settings.

0A6S1

Teaching practical skills in LAS – Best practice approaches and the use of teaching aides

Linklater Nicole
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Abstract
Handling of and performing procedures on animals are in most countries part of laboratory animal science (LAS) education and training. For participants in such training programs the achievement of practical skills is one of the main challenges. It is highly recommended to address such training in a two-tiered approach (first working with teaching aides and subsequently with live animals). Nevertheless, how and when to incorporate teaching aides or which ones depend on their availability but also on previous experiences and in some cases the ingenuity of the trainers too. Furthermore, the training may be influenced by different legal requirements in respect to the use of live animals in teaching and training as well as, for certain procedures, the research profile of the institution offering the training. In addition, the experience of the trainer or of the trainee play an important role in training for practical skills. One of the main goals for LAS courses/programs is to communicate and exchange these different approaches where possible. This way, a common best practice could be established across different LAS trainings.

0A6S2

''Start at the end and work backwards. . .'' How do you assess competence in procedures?

Whitfield Lucy
Named Veterinary Surgeons Group, Royal Veterinary College, London, United Kingdom

Abstract
It is essential that procedures involving animals are done competently and carefully, in order to ensure high quality, replicable science and to prevent unnecessary harm to the animal. But just how *do* we reliably assess someone's competence?

Often, the way to determine whether or not a person is competent in the task is for an experienced person to watch them and to make the judgement. So far so good. BUT: is this fair on every student? Does the 'trainee' know what you are looking for during this assessment and why you made your decision?

For persons who arrive already competent, how can you know the standard to which they were assessed (and working) in another unit? What is the ''right standard'' for a procedure?

What about Culture of Care? How can we incorporate those values into assessment and demonstrate that it really matters?

A good assessment must be fair (to the student), transparent (everyone understands what's going on) consistent (everyone is assessed to the same standard) and defensible (we must have a reason for our decision). This presentation will suggest ways in which you can achieve these goals for your practical assessments,

whilst promoting a supportive, learning culture and ensuring that there is sufficient resource to carry your assessments out!

Successful training starts with thinking first about the assessment at the end!

0A6S3

Use of simulators in veterinary education can help to increase animal welfare

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Abstract

Use of animals for educational purposes is a common ''teaching tool'' in several apprenticeships and university faculties. On the one hand legal and internal requirements of competences and skills are constantly increasing, expecially in the field of veterinary medicine and biomedical research and on the other hand public concerns about animal welfare in education are expressed. To meet these both concerns, potential harm and suffering of animals used in education and training should be minimized. Some basic knowlegde could be provided by images or films, computer simulations or organs from abbitoirs. Realistic simulators are an animal free alternative to gain manual skills. Advantageously the unexperienced learner's attention can be fully focused on the procedure, while making mistakes is allowed and a repeated carrying out may enhance the learning process without causing any additional suffering to any animal. Therefore, students might be better prepared when it comes to a first application to living animals and can easier concentrate on the patient. Some simulators are commercially available. At the Freie Universität Berlin new simulators and standard operation procedures for rodents, pets and farm animals are developed and evaluated in cooperation with students and integrated into curricular teaching.

The complete abandonment of live animals in education does not seem feasible, especially in professions that aim to work with living animals in the end, but the implementation of alternative methods for education, especially of realistic simulators might contribute to reduce the impairment of animals used for education.

0A6S4

Challenges and solutions to the delivery of training in practical skills in emerging countries

Bugnon Philippe

Institute for Laboratory Animal Science, University of Zürich, Zürich, Switzerland

Abstract

After visiting our basic education course in laboratory animal science for rodents in August 2014, Dr. Bert Mohr (Director at the UCT Research Animal Facility in Cape Town), contacted us three years later to organize a joint course to train people from all over South Africa to not only manage the basic course content but also to be able to develop their own skills as trainers. We accepted this challenge with Dr. Louise Martin from my team, Dr. Dave Lewis from the Faculty of Biological Sciences at the University of Leeds UK and with Dr. Emma Vodoti and Dr. Matti Nikkola, responsible for the Nordic Consortium for LAS Education and Training. With the support of the Basel Declaration Society we had previously trained future instructors in our courses but these were limited to one person a year. In May 2018 we faced the challenge to educate 33 trainers in state-of-the-art handling of laboratory rodents and in a second step how to impart that knowledge to their animal caretakers and scientist. The success of that week encouraged not only the future trainers but also all the international instructors to continue to find solutions to deliver education and training in newly industrialized countries.

0A7S1

Introduction of the FELASA-EFAT Working Group

Abelson Klas

Department of Experimental Medicine, University of Copenhagen, Copenhagen, Denmark

Abstract

The structure of education and training of animal caretakers and technicians varies considerably between European countries, both regarding the basic education and training and the continuous professional development (CPD). The current educational career pathways established by e.g. the European Federation of Animal Technicians (EFAT) and FELASA are not satisfactorily reflecting the diversity of the national educational pathways in Europe, nor are entirely updated with the EU Directive 2010/63/EU and the learning outcomes for EU function C in the adjacent Education and Training Framework. This means that the harmonisation of this education within Europe is obstructed, making a clear career pathway and organized continuous professional development (CPD) for European caretakers and technicians more difficult to obtain.

There is thus a need for a harmonised system for education, training and CPD that applies to all European caretakers and technicians to guarantee similar educational possibilities and facilitate mobility of these professionals. FELASA and EFAT have established a common working group with the task to scrutinize different educational and CPD programmes and career pathways for laboratory animal caretakers and technicians throughout Europe, and to write a recommendation for the harmonization of education and carrier possibilities for European laboratory animal caretakers and technicians. Besides harmonization, the recommendations are intended to form a platform for the establishment of new or adaptation of existing basic education, training and CPD activities.

OA7S2

National education schemes for animal caretakers and technicians: IAT

Fisher Glyn
Institute of Animal Technicians, London, United Kingdom

Abstract
The Institute of Animal Technicians (IAT) has been educating animal technicians since the 1960's and has established levels of qualifications from the moment a technician starts in the industry, then taking them through an industry recognised career pathway, to 1st degree level.

The IAT's education system moved in 2007 on to the QCF, allowing animal technicians to achieve an international recognised qualification, allowing animal technicians to develop in the workplace and take HE level qualifications if required.

During this presentation I will explain how an animal technician progresses through the career pathway, reviewing the educational levels and discussing the major learning topics and delivery styles to achieve the various qualifications.

I will also explain how the syllabus are structured to allow the student utilise their learning experience to then implement it in the workplace.

OA7S3

The Dual Vocational Training in Germany – A blessing and a curse?

Pohlig Paul Friedemann and Zevnik B.
in vivo Research Facility, CECAD- University of Cologne, Cologne, Germany

Abstract
Germany has a long-standing tradition in its ''dual vocational training'' which is unique in the international comparison. This dual vocational training system is based on compulsory schooling, including 13 years of education in general.

''Lab animal technician'' represents an excellent example for this dual concept in Germany. In general, this education comprises three years of training, split up in a part of vocational training school (1/3 of time, theoretical based) and on-the-job training in a company or institute (2/3 of time, practical based). Additionally, the trainee has to follow comprehensive practical courses, organized by all training companies and institutes together.

In spite of the great benefit, the practical as well as theoretical training of the young trainees of this dual training path uncovers disadvantages too. Indeed, the very strict and regulated curricula of the classical dual vocational education guarantees high quality levels, however, due to the vast restriction (up to 90%) to training with transgenic mice under access restricted SPF conditions, it is at the same time overly time intensive.

This negatively affects the capacity for educating trainees to meet the overall high demand for lab animal technicians, and often results in a situation, where the majority of animal facility personnel has other professional backgrounds and will receive an ''on-the-job'' training.

For this reason, our institute established an additional ''job training'' including a curriculum with more orientation to our individual needs.

OA7S4

Education schemes for animal caretakers and technicians in Scandinavia

Gorm Pedersen Lene[1] and Abelson K.[2]
[1]*Roskilde Technical College, Roskilde, Denmark*
[2]*Department of Experimental Medicine, University of Copenhagen, Copenhagen, Denmark*

Abstract
FELASA and EFAT (European Federation of Animal Technicians) have established a common working group with the task to scrutinize different educational and CPD programmes and career pathways for laboratory animal caretakers and technicians throughout Europe, and to write a recommendation for the harmonization of education and carrier possibilities for these professionals. In order to make this possible, a scrutiny of existing educational schemes around Europe is necessary.

In the Scandinavian countries Denmark and Sweden, the education of animal caretakers is well structured and is organized as an education at high school level or similar, lasting for 3-3,5 years. After graduation, the caretakers are qualified at a level fulfilling the learning outcomes for the core and function specific modules for functions A, C and D, and to some extent learning outcomes for the additional task specific module on advanced animal husbandry, care and enrichment practices (module 23), according to the Education and Training Framework established by the EC expert working group in February 2014.

This presentation will give an overview of how the education schemes in the Scandinavian countries (with primary focus on the Danish system) have been structured in order to qualify the students at the above mentioned level.

OA7S5

Contribution of AFSTAL in the education and training of technicians in France and around

Vidal Samuel[1], Bouchoux E.[2] and Jacquot F.[3]
[1]*VetAgro Sup, Biovivo, Marcy l'Etoile, France*
[2]*Sanofi Recherche et Développement, Sanofi, Chilly Mazarin, France*
[3]*AFSTAL, Association Française des Sciences et Techniques de l'Animal de Laboratoire, Paris, France*

Abstract
AFSTAL – Association Française des Sciences et Techniques de l'Animal de Laboratoire – was originally founded in 1972 as ''Société Française d'Expérimentation Animale''- SFEA French Society for Animal Experimentation).

Technical training and promotion of best practices towards technicians are conducted more specifically through the following ways:

- The publication of a quarterly magazine, STAL, written in French. This journal is very accessible and it welcomes every proposition of scientific and technical article. Any expert, technician or technical team can share and promote in vivo technique illustrating 3Rs implementation or innovation. The journal also publishes international recognized articles in French language to facilitate knowledge dissemination among technicians.
- With its technical committee ComTech, AFSTAL supports a very active group of technicians and experts in animal facility management. This group organizes every year a very successful one-day congress, where nearly 500 technicians can attend a high quality conferences program at a low price, and at an easily accessible venue in the center of Paris. ComTech is also promoting personal development. This group edited a ''Competences passport'' which was largely distributed to AFSTAL members for the follow-up of competences in laboratory animal field.
- Finally, since 2007, AFSTAL has organized thematic free training workshops, in which specialized topics are taught by volunteer professionals. Once a workshop is set up, it starts a ''Roadshow'' all around France and francophone countries (Switzerland, Belgium). Examples of workshop themes were: Animal Welfare Bodies, Animal Nutrition, Rodents health monitoring, Genetically modified rodents, etc...

OA8S1

Integrity, Communication and Respect Exemplifies ICARE

Boden Tania
UCB, Slough, United Kingdom

Abstract
The Institutional Care and Animal Welfare Responsible (ICARE) should influence and drive the culture of care within an establishment, this will not only lead to better animal welfare but also improve scientific outcomes. Understanding and respecting what colleagues are trying to achieve is essential to effect change and make a difference, it also helps to unify the desired culture. Discussing experiments before they begin and possible adverse events can lead to mitigating welfare issues or avoiding them entirely. When unexpected events occur, this collaborative background can help to clarify problems and improve further studies. Therefore, consistently and appropriately we should be asking ourselves and others, ''How can things be done better?''

OA8S2

Problems and solutions in the performing with CO2 as an Euthanasia agent in mice

Pohlig Paul Friedemann[1], Wecker S.[2] and Zevnik B.[1]
[1]*in vivo Research Facility, CECAD- University of Cologne, Cologne, Germany*
[2]*medres – medical research GmbH, Cologne, Germany*

Abstract
With the opening of our new large mouse facility within a research centre (CECAD- Cluster of Excellence in Cellular Stress Responses in Aging-Associated Diseases) in 2013 we dealt intensively with the subject of euthanasia, since killing of animals was not carried out solely by the staff of the animal unit, but also by researchers.

Use of carbon dioxide (CO_2) or cervical dislocation are the main methods employed for euthanasia of mice.

Even if trained in mouse euthanasia, people often feel unsure how exactly to comply with CO_2 euthanasia procedures. However, wrongdoing or misusage is likely to cause immediate stress and pain for mice.

First we used a system to assure a constant flow rate and slow increase of CO_2 in purposely designed chambers. A remaining issue was, that we could not guarantee the required increase of CO_2 due to the design of the chamber.

Therefore, the procedure was purely visually monitored for signs of stress and pain in mice.

In order to prevent possible misuse, we developed in cooperation with medres a fully automated fail-safe solution that guarantees a constant and measurable increase of CO_2, in which we use the air flow of the existing IVC cage that fully complies with our and the requirements of the ''AVMA Guidelines for the Euthanasia of Animals 2013''.

OA8S3

A working example of murine cryopreservation and rederivation whilst maintaining aseptic technique

Newman Stuart[1,2]
[1]*Biological Services, King's College London, London, United Kingdom*
[2]*Antibody Innovation in Animal Health, PetMedix Ltd, Cambridge, United Kingdom*

Abstract
Distribution of defined mouse models around the world has been driven through collaboration and the availability of material from large scale repositories. The shipment of cryopreserved material has also been championed due to the unethical nature of exporting live animals, particularly over long distances. The ability to generate and recover from cryopreserved material permits great local control over mouse exports and imports.

During 2015–2016 a full range of embryology services were developed within King's College London Biological Services which helped refine the exportation and rederivation of mouse models. Critically this involved good aseptic technique whilst performing surgical implantation of embryos. This has been of upmost importance for both animal welfare and for maintaining biosecurity for mouse lines entering into high health status research facilities.

This presentation will address the practices required to action the cryopreservation, recovery and embryo implantation whilst maintaining good aseptic technique. Working examples of how aseptic technique has been refined will also be included.

The implementation of such robust working practices has enabled establishment-wide cryopreservation projects, large scale rederivation projects and provides effective local control of ethical mouse model distribution.

OA9S1

Good practice in mouse handling

Hurst Jane
Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom

Abstract
The traditional method of picking up mice by the tail induces aversion and high anxiety. Mice do not readily habituate to tail handling, even if their weight is supported and the duration is brief. This negatively impacts animal welfare, and can also interfere with reliable test responses. We have shown that picking up mice in a tunnel or scooping them on the open hand (cup handling) are refined methods that mice accept much more readily, particularly tunnel handling. But are these refined methods practical for general use in the laboratory? Here I will discuss good practice in using tunnel and cup handling for laboratory mice, the training that is required to use these techniques efficiently and resources that are available to help with this. I will discuss the costs and benefits of each method, barriers to uptake, and recommend some practical approaches to aid the implementation of these refined methods in busy animal facilities. Finally, I will report feedback from some of the facilities that have already implemented refined mouse handling.

OA9S2

Tube versus tail handling: no impact on spontaneous activity in C57BL/6J female mice detected

Martijn Bolderheij and Prins J.
Leiden University Medical Centre, Leiden, Netherlands

Abstract
Since 2010 there is increasing debate about the pros and cons of tube handling of mice compared to tail handling. The debate was fuelled by the paper by Hurst and West (1) in which they reported that picking up mice by the tail induced high anxiety, whereas use of tunnels or so called 'cupping' led to less anxiety. In another publication it was reported that tube handled mice performed more reliably in behavioural tests than tail handled mice (2). We recorded the spontaneous activity of female C57BL/6J mice (12 weeks old) in a crossover experimental design study. For this purpose, animals were housed in a DVC® housing system (Tecniplast S.p.A.), which registers 24/7 home cage activity. Mice ($n = 32$) were housed in pairs and randomly assigned to 16 cages. Cages were randomly distributed over the rack and randomly assigned to handling method. Mice in 8 cages were tube handled or tail handled three times per week for a period of 6 weeks. The following 6 weeks tube handled animals were tail handled and vice versa. We did not detect an impact of handling method on the spontaneous activity of the mice. However, a period effect was evident with greater activity in period 2 compared to period 1, which could not be explained by a change in environmental factors. The latter finding illustrates the importance of systematic monitoring of different endogenous and exogenous variables around the rack, that potentially affect the mice while in their cage during normal housing practices.

OA9S3

The transition from open-top conventional cages to individual ventilated cages (IVC)

Woodley Stephen
Biological Services, King's College London, London, United Kingdom

Abstract
The demand for modern animal facilities is an increasingly important area for change within in vivo research. The use of IVC environments enables a reduction in variability to research by providing a stable bio-secure environment by reducing the risk cross-contamination, allow for increased cage density and facilitate improved health and safety by reducing levels of laboratory animal allergens (LAA).

From 2015 to 2018, King's College London, Biological Services New Hunt's House, a facility of 900 sq. m. holding 11,000 mice, transitioned from a variety of mouse caging environments (open-top, Scantainers, and IVC) to a cohesive environment of a single type of IVC caging. During this period there was also a large-scale in-house rederivation programme to provide a high-quality defined health status within the facility (SOPF+), eliminating several endemic pathogens, including MHV and pinworm.

This presentation will discuss the application of the practical processes required within a large-scale animal research facility, discussing the importance of project management, sign-up by all the staff, both animal technicians and researchers to deliver these changes, whilst maintaining normal business operations.

Following the successful delivery of these changes, we have seen improvements to animal welfare, reduction in scientific variability, particularly for behaviour studies, cleaner and leaner working environment, improved practice for movement of animals across multiple sites and a proven reduction to LAA through scientific tests.

OA10S1

Training procedures for staff using farm animals for scientific purposes: farm level, transport, slaughterhouse

Marinou Katerina[1], Sossidou E.[2] and Doudounakis S.[1]
[1]*Animal Welfare for Farm and Laboratory Animal, Ministry of Rural Development and Food, Athens, Greece*
[2]*Veterinary Research Institute, Hellenic Agricultural Organisation – DEMETER, Thessaloniki, Greece*

Abstract
According to the Directive 2010/63/EU on the protection of animals used for scientific purposes, Member States shall ensure that each breeder, supplier and user has sufficient staff on site which is adequately educated and trained before they perform animal studies. In the case of farm animals, education and training should include not only laboratory facilities but also farm, transport and slaughterhouse facilities, where relevant research takes place. Relevant training programs are currently organized by competent authorities or education and training bodies and organisations on topics of welfare at farm level, at slaughterhouses and during commercial transport. The personnel should be trained under institutional responsibility both regarding its species specific needs, but also husbandry and welfare needs, as well as recognition of signs of pain and illness. Courses should be designed to meet the particular needs of researchers, breeders and animal care staff with the scope, detail and content reflecting the responsibilities of each. Practical sessions should be scheduled as required and, as much as possible, be focused on the needs of individual participants which may be species and procedure specific. Keeping of training records by the designated veterinarian and/or responsible scientific personnel is strongly recommended to be included in course contents. Moreover, institutions may consider ways in which they can assess whether or not the students have achieved the learning outcomes of a particular course or course component. Finally, the didactic methodology should be chosen to suit the module and the learning needs of the target audience.

OA10S2

Minipigs: vascular access and welfare

Zeltner Adrian
Ellegaard Göttingen Minipigs, Dalmose, Denmark

Abstract
Reliable non-invasive methods to assess welfare and species-specific benchmarks need further development in Minipigs. Unfortunately, no single golden standard exists for welfare assessment. As in all other cases, a profound understanding of biology and behaviour of the specie in question is essential to assure good welfare. Needs must be distinguished from preferences, if needs are not adequately met it will be characterized by measurable welfare impairment, as not meeting preferences may not have the same impact.

In this presentation examples will be given how, sometimes with little investment of money or time, a big improvement in the Minipig welfare can be achieved. The focus however will be on procedures, namely vascular access. Serial infusion and blood sampling are often important technical aspects of an experimental design. Superficial vessels in the minipig are not readily accessible. Although minipigs have a convenient size for handling, restraint and venipuncture can be stressful and affect blood parameters. Ethically and scientifically a catheter might be appropriate, but the implantation process and housing afterwards might have an influence on welfare. Sometimes compromises must be made and different factors that influence welfare have to be balanced against each other. Another aspect in welfare that is not much talked about is cognitive enrichment. There are publications but in praxis it does not seem to be applied very often. A short introduction is given to encourage further exploration in that field.

OA10S3

New world camelids: Implementation of the 3Rs in a research farm

Füner Jonas, Dühlmeier R. and Ferrara F.
Pet and Farm Animal research department, Preclinics GmbH, Potsdam and Campus Eystrup, Germany

Abstract
Introduction: New World Camelids (NWC) are frequently used for immunisation trials, e.g. to generate nanobodies for different research topics. A special feature at Preclinics Research Farm is the immunization of llamas and alpacas for the production of heavy chain antibody fragments VHH and the breeding as laboratory animals. Naturally, NWC are herd- and running animals. Consequently, despite the high requirement of personnel expertise, housing NWC is also challenging due to the large space requirements. In accordance with the legal requirement to implement the 3R principle (European Union directive 2010/63), it is of high importance to implement species-specific measures to ensure or improve animal welfare through better housing facilities and handling practices.
Methods and Results: At the Preclinics Research Farm housing of NWC, the obligatory welfare requirements included daily access to large pasture areas and group housing. We additionally implemented measures, such as the provision of sand baths and elevated areas, to improve animal welfare. We also developed a strategy to minimise the stress of immunisation and blood collection procedures, which lead to the prolonged use of each NWC in our immunisation trials. Importantly, the impact of well qualified animal caretakers is very crucial to develop and ensure animal welfare strategies.
Conclusion: To implement the 3R principle within the animal housing and within the procedures it is necessary to evaluate and implement the species-specific needs and the impact of animal caretakers.

OA10S4

Large animal housing and welfare in containment facilities

Garthwaite Joe
Animal Services, The Pirbright Institute, Pirbright, United Kingdom

Abstract
Infectious disease research in farm animal species has a number of unique challenges when compared to non-infectious disease research in ''traditional'' laboratory species.

The animal species housed at The Pirbright Institute include Cattle, Sheep, Pigs, Goats and Chickens. These each have their own environmental, nutritional and behavioural needs, which must be optimised to maximise animal welfare. These needs, however, must be delivered whilst ensuring the highest levels of biosafety – a most important factor when working with highly contagious viral diseases such as African Swine Fever and Foot-and-mouth disease in CL2-CL4 animal facilities.

In this presentation, an initial overview of the construction and principles of working in a high containment animal facility will be given using examples of the major engineering and procedural controls respectively. Then approaches to housing livestock in such an environment will be given which will include: 1) Strategies of enrichment provision to allow animals to exhibit natural behaviours, 2) Approaches to monitoring animals to allow retrospective assessment of any health problems to be investigated, 3) New ways of quantifying the behaviour in order to produce evidence based methods and approaches to housing animals in such facilities.

Delivering a more enriched environment, and therefore enhanced life experience, for the animals used in infectious disease research provides a foundation for the scientists to achieve more consistent results from the calm and contented animals. All Animal Technicians will also tell you that a happy cooperative animal makes for a happy technician!

OA11S1

Training the Trainers – Ensuring standardisation and competency

Garrod Ian
Learning Curve (Development) Ltd, Ware, United Kingdom

Abstract
The objectives are to ensure we have a systematic approach to training delivery and how to effectively deliver a participative training session, understanding learning processes and recognition of individuals learning preferences. This should result in successful training and demonstration of skills learnt both during the training session(s) and subsequently in the workplace.

The presentation will cover important hints and tips for both new and established trainers to ensure both standardisation in training but also ensuring trainers feel competent and confident in the delivery of their training.

Within the presentation the following topics will be discussed:

- What makes training effective
- Planning and preparation
- Trainer teaching styles
- Question techniques
- Learning for the senses
- The Learning Process

OA11S2

What is a ''good exam?'': Learning outcomes, education & assessment of students

Whitfield Lucy
Named Veterinary Surgeons Group, Royal Veterinary College, London, United Kingdom

Abstract
We all have our own views – and experiences – of examinations and this can colour our approach to this subject, whether as trainee or when designing exams. So, forget your fears... take a deep breath and let's take a dispassionate view of assessment, training and the people involved!

By taking a student-centred approach, we consider what it is that the student needs to know, or be able to do, at the end of our module of training – and this 'Learning Outcome' is what we need to assess. As educators, we should then design the assessment task to match this output.

Learning can have one of three aspects: knowledge & understanding (eg factual recall), skills (eg doing a task) and/or attitude (behaviours) and the assessment should align with these areas. Layered onto this, the three areas can be simple or complex (eg basic factual recall vs high-level analysis) and similarly, our assessment should reflect the level at which we ask the student to perform.

Learning outcomes and assessment are inextricably linked and a well-designed assessment will enable us to determine how well the student has achieved our intended standard. The assessment must be valid and reliable each time that we run it but we also have to ensure that the assessment is feasible with the resources available. Lastly, assessments should have a positive educational impact, to encourage lifelong learning in our students . . .

So – remembering your own experience – how shall we enhance exam design these days, to optimise output and leave better memories?

OA11S3

How to set-up a good assessment in view of competence-driven education?

Eyckmans Marleen and Van Ginneken C.
University of Antwerp, Antwerp, Belgium

Abstract
In this part of the session, you will learn how to use and adapt a simplified examination matrix to your own course. By defining your course competences and learning outcomes and looking at the importance of each course competence and learning outcome in

the totality of the course you are well on your way to have a clear and schematic overview of how to evaluate your students.

Connect this to which format you use to teach these competences and to which format and criteria you use to assess these competences and it will be much easier to detect if there is a good match between the assessment and the learning outcomes you are evaluating. Finally, by linking the course competences to a certain level of complexity/specificity and to the overall core competences of a e.g. master program, you are able to position your course and its assessment in the competence-driven education of most higher education institutions.

OA12W1

Are you skilled enough to work with animals? Measuring competencies using OSCE stations

Costa A and Olsson Anna
i3S – Institute for Research and Innovation in Health, University of Porto, Porto, Portugal

Abstract
The terms competence/competent are used in Directive 2010/63/EU as a requirement for persons carrying out procedures with animals. More recently, several initiatives have started to address the question of competence is to be assessed in order to guarantee that future animal users are skilled enough to be authorized to work without supervision.

Following the Objective Structured Clinical Exam (OSCE) approach, we have developed an instrument to assess students' ability to perform handling and key procedures with rodents (restraint and two types of injections).

The process of development and definition of the marking rubric was initially based on revision of the standard reference material and experts' contribution – trainers in practical classes, technicians and the responsible laboratory animal veterinarian. Within this process, measurable and crucial steps for the success of the procedures were identified and a consensus for approval criteria was established.

Subsequently, the instrument was tested in a pilot evaluation context, to check that the itmes were adequate and operational in a practical setting.

The third step in this development process was the incorporation of a number of changes based on specialists' revision; items were eliminated and categories condensed and a final score sheet for each procedure was established with a 3-point answering scale (from *fail* to *clear pass*).

In this workshop, participants will be given an introduction to the concept of OSCE and how to develop them, and thereafter get the opportunity to practice scoring different student performances from video recordings.

OB1S1

Reproducibility: ''10% of the time it works every time'' – Time to take stock

Begley Glenn
CEO, BioCurate Pty Ltd, Parkville, Victoria, Australia

Abstract
As medical researchers, we all want to make discoveries that have a real impact on human health. There are however serious obstacles preventing this.

A key impediment is the reward system that applies within academic research, and that provides a perverse set of incentives that reward flashy science with little regard for the quality, robustness or reliability of the work. That is particularly the case for papers published in the ''top tier'' journals. Those highly cited publications, from famous investigators typically fail because experiments were not performed by blinded investigators, positive and negative controls were not used, experiments were not repeated, reagents were not validated, only select data was shown, and data analysis was inappropriate.

Over recent years, positive steps have been taken to begin to address this issue. Some funding agencies and a number of journals have sought to improve their processes.

However, the principal responsibility for addressing this problem rests with investigators and research institutions.

Researchers can actively foster quality research: scientists should actually read papers before citing them, refuse to cite poor quality papers (even from famous investigators), refuse to accept the journal as a surrogate for quality, and do things properly ourselves.

Research institutions should create an ''Office of Research Integrity'', with responsibility for conducting random laboratory audits, compulsory methods-training, requiring compliance with agreed Guidelines. Failure to comply should be associated with real consequences.

These would be important steps to help address this important, systemic problem.

OB1S2

How standardization causes poor reproducibility in animal research

Wuerbel Hanno and Voelkl B.
Division of Animal Welfare, University of Bern, Bern, Switzerland

Abstract
Reproducibility in animal research is alarmingly low. Various potential causes of poor reproducibility have been identified, including poor scientific rigor, low statistical power, analytical flexibility, and publication bias. However, the reproducibility of a result is also a function of its external validity. Unless results are robust against common sources of variation between independent replicate studies, they will not be reproducible. In animal research, effects of experimental treatments usually vary depending on the phenotype of the animals. Because the phenotype depends also on the environment of the animals, small differences in the environment between replicate studies can produce conflicting results.

Therefore, systematic variation (heterogenization) rather than more rigorous standardization is needed to improve reproducibility. Based on a theoretical analysis of phenotypic plasticity, simulations with existing preclinical animal data, and experimental results, I will show how heterogenization of study populations can improve robustness and reproducibility of results without a need for lager sample sizes. Using more representative study samples will be crucial to avoid wasting animals for inconclusive research.

OB1S3

Promoting transparency in preclinical research: Preregistration of animal studies on www.preclinicaltrials.eu

de Leeuw Wim[1], van der Naald M.[2], Wever K.[3], Duncker D. J.[4,5], Chamuleau S.[2] and Smit N.[5]
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[2]*Department of Cardiology, Division Heart and Lungs, University Medical Center Utrecht, Utrecht, Netherlands*
[3]*SYRCLE, Radboud Medical Center, Nijmegen, Netherlands*
[4]*Experimental Cardiology, Erasmus Medical Center, Rotterdam, Netherlands*
[5]*Netherlands Heart Institute, Utrecht, Netherlands*

Abstract
Background: Publication bias, selective outcome reporting, and risks of bias limit the validity and reproducibility of animal studies and threaten the translational value. Preregistration is a promising process to reduce these limitations and create transparency. For clinical trials preregistration is standard however, this is not the case for animal study protocols, whilst these form the basis for clinical research. On preclinicaltrials.eu we have developed an online platform to preregister animal study protocols.
Objectives: An expert group on preclinical evidence synthesis designed the registration form which consists of 34 fields. Details of the study's hypothesis, design, outcome measures (primary and secondary), measures to reduce bias and sample size rational are asked for. Authors need to indicate whether their study is exploratory or confirmatory. Reference to publication(s) or data repositories can be provided. Protocols can be made publicly directly after submission, or after an embargo period. Preclinicaltrials.eu aims to provide an overview of all executed animal studies, including those that remain unpublished, and thus contribute to a reduction of publication bias and unnecessary duplication. It allows reviewers and researchers to access additional information on the study design. Finally, preclinicaltrials.eu aims to increase awareness and transparency concerning risk of bias and selective outcome reporting and potentially reduce these biases.
Future perspectives: We believe all stakeholders involved in animal research should encourage preregistration and call upon researchers, institutes, medical journals, funding bodies, policy and law makers, scientific societies and others involved parties to make prospective registration the standard and mandatory in animal research.

OB2S1

Implementing humane endpoints in a mouse model of fatal neurodegenerative disease

Grierson Andrew
Department of Neuroscience, University of Sheffield, Sheffield, United Kingdom

Abstract
Amyotrophic lateral sclerosis (ALS), known commonly as motor neuron disease, is a fatal neurodegenerative disease with no effective treatment. Patients typically present in middle age with weakness in one limb, and once diagnosis has been confirmed clinically, progress towards paralysis and death with a mean disease duration of about 2 years.

Around 20% of ALS cases are inherited, and mutations in a number of genes have been identified as causative. Cellular and animal models are used to identify disease mechanisms and test potential therapies. The most commonly used mouse model (>200 publication per year since 2009) is a transgenic mouse overexpressing mutant SOD1. The majority of these studies involve a specific G93A SOD1 transgenic line that develops stereotypical disease progression involving onset of hindlimb paralysis at 3 months of age and loss of self-righting ability 3-4 weeks later.

For preclinical studies testing therapeutic approaches in G93A SOD1 mice, loss of self-righting ability is a commonly used endpoint. This means that the majority of mice used for this purpose will experience severe distress involving progressive paralysis. To address this we have optimised the use of early disease readouts using a combination of gait analysis, rotarod testing and home cage running wheels. These tests are sensitive and utilise small group sizes, thus enabling affordable large-scale therapeutic prescreening. This paradigm is now implemented in our Institute as the standard preclinical screening platform for G93A SOD1 mouse studies.

OB2S2

Humane endpoints in sepsis models

Drechsler Susanne
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Abstract
The implementation of adequate humane endpoints to reduce the suffering burden for animals in preclinical sepsis studies remains problematic and death remains the most frequent endpoint that is used in sepsis models. The fact that the complex underlying pathophysiology of sepsis varies strongly in relation to its severity has long impaired creation of uniform criteria for humane endpoints and euthanasia. Available humane endpoints are based on body temperature monitoring and/or altered behavioral parameters and clinical symptoms. However, although they help to eliminate death from an experiment, they do not reduce the suffering that occurs prior to the impending demise.

Complete elimination of the death endpoint based on commonly available humane endpoints remains controversial because it may

lead to a loss of important information regarding the experimental outcome. Furthermore, pre-emptive euthanasia may result in erroneous outcome assumptions that promote incorrect conclusions from the collected data. This ultimately impairs the animal-to-human translatability and is incompatible to human sepsis trials in which mortality endpoint is frequently used.

While we refine already existing endpoints, the search for new indicators/surrogates of sepsis-induced morbidity and mortality is ongoing, e.g. by using biomarkers and telemetry devices for vital sign monitoring. The ultimate goal is to determine earlier humane endpoints that reliably predict death and long-term survival of septic animals.

OB2S3

Humane endpoint – Concept and misconceptions

Brønstad Aurora
Department of Clinical Medicine, University of Bergen, Bergen, Norway

Abstract
Humane endpoints are a refinement strategy to prevent unnecessary pain, suffering and distress of research animals. Defining humane endpoints often involves establishing the upper threshold of harm necessary to achieve a certain legitimate benefit, when designing and planning experiments, considering both ethical and scientific arguments. The directive 2010/63/EU explicitly states that death as an end-point shall be avoided as far as possible. However, it should also be carefully evaluated in which cases near-death, severe clinical statuses should be acceptable as endpoints, as they often result from unameliorated and prolonged pain, suffering or distress, thus also in potential violation of the Directive.

This presentation will include some examples of sound humane endpoints and implementation strategies, as well as discuss some common misconceptions in the application of humane endpoints.

OB2S4

Humane endpoint – The designated veterinarian's perspective

Glage Silke
Instituite for Laboratory Animal Science, Hannover Medical School, Hannover, Germany

Abstract
The revised Directive 2010/63/EU requires the European Union Member States to implement the Three R's principles in their national laws. Therefore researchers have been legally required to reduce any pain, suffering and distress in laboratory animals during an experimental procedure to a minimum.

Effective alleviation of pain, suffering and distress is highly depending on the ability of researchers to recognise this and to assess its severity. It is recommended to develop a specific and reliable score sheet for each procedure to recognise pain and distress and to define humane endpoints.

A humane endpoint does not necessarily mean the humane killing of the animal and the end of a procedure, but could also result in interventions to alleviate the pain and distress (decision-making model) and therefore helps to limit the severity of a procedure.

Developing an appropriate score sheet that considers all aspects of a scientific model necessitates a good cooperation between scientists and the designated veterinarian.

Crucial for the applicability of such a score sheet is the definition of criteria that are obvious and easy to assess. Despite the classical clinical parameters, physical and biochemical parameters are available. In addition, efforts are taken to define new behavioural tests for severity assessment.

In this lecture, a brief overview of classic and new criteria for the severity assessment and definition of humane endpoints with respect to their applicability and feasibility in a laboratory working routine is given from the designated veterinarian's perspective.

OB3S1

Hot topics & heated debates: Impact of animal husbandry environments on rodent thermoregulation

Hankenson Claire
Campus Animal Resources, Michigan State University, East Lansing, United States

Abstract
Housing room temperatures for laboratory rodents, as described in the U.S. *Guide for the Care and Use of Laboratory Animals,* are believed to have been established for the comfort of humans working in the facilities. Over the last two decades, laboratory mice and rats have been shown to prefer temperatures that are several degrees warmer than the environments in which they are typically housed in biomedical facilities. Physiological changes to rodents housed under 'cold stress' in modern vivaria include alterations in metabolism, cardiovascular function, respiration and immunologic function. Various disciplines of medical investigations, including cancer models, atherosclerosis, liver disease and microbiome research, have published on blunting of disease phenotypes when mice are housed under *Guide* conditions (20–26°C). While cage accommodations can be made to include warmth through increased stocking density, nesting materials and shelters, these may not be sufficient to result in consistent and reproducible data outcomes. Within the animal research industry, creative approaches to warming sections of cages and racks or increasing room temperatures are being explored. Relatively innocuous laboratory animal practices may well contribute to the inconsistency of murine models of human disease outcomes; biases may be introduced through provision of thermal support, selection of certain pre-surgical skin preparation agents, management of temperature and humidity vacillations due to HVAC and equipment failures, and housing practices. Environmental and procedural interventions for laboratory mice and rats will be discussed relative to the importance of achieving reproducibility and repeatability of rodent studies.

OB3S2

Metabolic consequences of individual housing in male C57Bl/6J mice after weaning

Schipper Lidewij[1,2], van Heijningen S.[2], Karapetsas G.[2], van der Beek E.[1,3] and van Dijk G.[2]
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[2]*GELIFES, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, Netherlands*
[3]*Department of Pediatrics, University Medical Center Groningen, Groningen, Netherlands*

Abstract
Rodents studies which model human disease and/or treatments differ widely in study design, with varying (social) housing conditions depending on e.g. type of data collected and local husbandry practices. While individual housing can lead to chronic psychosocial stress it also prohibits social thermoregulation. Together, these factors may modulate metabolic health. In the current study we have characterized the metabolic consequences of individual vs social housing of male C57Bl/6J mice.

Mice were housed either individually (IND) or socially (SOC; $n = 2$ siblings/cage) in a temperature-controlled room ($21 \pm 2^{\circ}C$) from weaning onwards. Body weight (gain) was monitored and energy intake and expenditure were determined using indirect calorimetry during adolescence and adulthood, under normal and (adult) high fat diet conditions. Femur length, body composition and plasma hormones were determined at 6 and 18 weeks of age.

Individual housing increased caloric intake and energy expenditure. While growth rate was reduced during adolescence, weight gain of IND exceeded that of SOC in adulthood. At both life stages, however, IND showed higher adiposity and reduced bone length compared to SOC, with plasma hormones matching this phenotype. Adult high fat diet exposure further amplified these differences.

In conclusion, individual housing of male mice under standard laboratory temperature affects energy balance regulation and metabolic health outcomes. These factors have the potential to influence other experimental outcomes used in mouse models of human disease. Increased awareness and understanding of the metabolic consequences of rodent housing practices can support reproducibility and translation of study results.

OB3S3

Diurnal rhythm and design

Nevalainen Timo
Laboratory Animal Center, University of Eastern Finland, Kuopio, Finland

Abstract
The animal life follows a variety of cycles; one of them being the diurnal rhythm. This 24-hour rhythm governs many aspects of animal physiology, and changes seen are major variations around the mean. The rhythm has an endogenous nature but it can be derailed e.g. by changing photoperiods or by restricted feeding. Since the diurnal rhythm is part of normal physiology, anything that disrupts it should be avoided. Among the parameters with considerable diurnal rhythm are e.g. melatonin, total fatty acids, glucose, lactic acid, corticosterone, insulin, and leptin. Scientists should always find out whether their parameter shows considerable diurnal rhythm. Diurnal rhythm is a challenge to study design; ignoring the rhythm is likely to cause increased variation and thus prevent statistical significances. Prolonged sampling or recording during one day, even in randomized order, exposes the study to decreased precision. Instead, one should block the experiment into smaller parts each lasting a few hours, and sample or record each block on separate days, but at the same time of the day. Using sample diurnal rhythm curves, calculations have been done to give metrics on a variety of scenarios. Depending where on the curve the sampling window is placed and how wide the window is, our calculations show that increase in precision, compared to ''as long as it takes'' approach, can be huge. Coping diurnal rhythm with narrow sampling or recording window is an important reduction method achievable with study design.

OB3S4

African mole-rats: A new model of laboratory animals entering the scene

Sumbera Radim
Zoology, University of South Bohemia, Ceske Budejovice, Czech Republic

Abstract
In recent years, there has been a high need for new mammalian models in biomedical research, other than laboratory mice or rats. One of the most promising species fulfilling this role is the naked mole-rat, *Heterocephalus glaber* belonging to a family of strictly subterranean African rodents (African mole-rats, Bathyergidae). This hairless small rodent has become the centre of several biomedical researches such as longevity, cancer resistance, pain insensitivity and resistance against hypoxy in last 15 years. Interestingly, recent findings have demonstrated that some other mole-rat species share many of these pronounced adaptations and even that some of these characteristics evolved convergently in phylogenetically unrelated subterranean rodents such us the blind mole rat of the genus *Spalax*. It is highly probable that most of these highlighted phenomena are consequences of staying in a very particular ecotope for whole life. This subterranean environment is dark, deprived of most sensory cues, very humid and also hypoxic and hypercapnic in some situations. Mammals living here are adapted to massive digging in order to obtain enough food, sexual partners and to disperse. Specific conditions of subterranean burrows is the main obstacle to keep these mammals in captivity due to difficulties with simulating such conditions. In our lab, we carry out behavioural and physiological research on seven species of subterranean rodents, especially African mole-rats, representing different phylogenetical lineages, ecologies, body masses and degrees of social organisation. In my talk, I will review 20 years of our experience in keeping, breeding and studying these highly specialised mammals.

OB4S1

LA-day on lab animal research reporting

Riederer Beat M.
Editior-in-Chief LA; c/o Plateform of Morphology and Department of Psychiatry, University

Abstract
Hospital of the Canton Waadt (CHUV) and University of Lausanne, Lausanne, Switzerland

In the light of recent developments of how to improve research reporting, it becomes evident that current guidelines such as Gold standard publication checklist, the ARRIVE-guidelines, and recently published PREPARE-guidelines, need reinforcement and implementation in current practice. Therefore, the LA-day tries to underline the importance of reporting research at all levels, planning, execution and reporting research data. Such guidelines are important to improve publication of research data, but may help at early stages of research, such as review of relevant literature. At the center for any research project is the planning of experiments and optimal experimental design and formulating a working hypothesis. This is as well essential for preparing grant proposals, for funding, for obtaining appropriate licenses and ethical approval. Furthermore, it favors research reporting of preclinical research and publishing research data, which in turn primes funding. The LA day is organized in four sessions that focus on knowledge of literature, on experimental design, on guidelines and scientific reporting, on sponsoring and editorial aspects, on publication of laboratory animal research data and on ethical concerns in publishing animal welfare data.

The LA Journal is the official Journal of FELASA and printed on behalf of LAL. This event is sponsored by LAL.

OB4S2

Systematic reviews of preclinical studies

Ritskes-Hoitinga Merel
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Abstract
ZonMw, the Netherlands Organisation for Health Research and Development, has funded synthesis of evidence programmes in the form of teaching and coaching of systematic reviews of animal studies at the SYstematic Review Center for Laboratory animal Experimentation (SYRCLE). The aim is to increment the quality of research for the benefit of both animals ánd human patients. By facilitating guidance on performing systematic reviews of animal studies, awareness is created on how to enrich research when doing animal studies, e.g. to improve translation to humans.Teaching systematic reviews of animal studies takes place in one-day hands-on workshops and providing coaching to participants during the course of performing their own systematic review. From 2012 to 2017,16 one-day workshops were held with ~300 participants. Participants rated workshops 8 or higher (10 point scale). A total of 55 systematic reviews were coached by SYRCLE scientists within the ZonMw programme. Nearly half of these have already been completed/published, the others are still being conducted. Results of these reviews clearly demonstrate where value in research can be increased: comprehensive search of all relevant literature, more evidence-based selection of suitable animal models, transparency on quality of reporting and translation, and summary of the evidence of animal studies before embarking on clinical trials.

OB4S3

PREPARE before you ARRIVE: Good reporting relies on good planning

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[4]*Norwegian University of Life Sciences, Oslo, Norway*
[5]*Western Norway University of Applied Sciences, Bergen, Norway*

Abstract
Poor reporting, despite extensive endorsement of the ARRIVE guidelines, is often highlighted when the poor reproducibility of animal research is debated. Improved reporting is frequently promoted as the most important method of raising standards.

There is an elephant in the room, however, which has received too little attention. Reporting guidelines cannot improve the intrinsic quality of experiments which have already been performed. Systematic improvement of animal research must begin with guidelines for *planning* experiments. This approach will also advance the implementation of the 3Rs.

The PREPARE guidelines for planning animal research and testing (https://norecopa.no/PREPARE) aim to address this need. Although they contain many of the elements mentioned in reporting guidelines, they also highlight issues which are seldom reported. Many of these can have major effects on the scientific validity of the research, animal welfare and the safety of all those involved. They are based on over 30 years' experience in planning and supervising animal research.

PREPARE has a 15-point checklist, but more importantly, the website offers scientists links to the latest guidelines for each topic. An intelligent search engine, on the 7,500-page Norecopa website where PREPARE resides, facilitates the location of additional resources.

We hope that the debate about reporting will rotate more towards the planning of animal experiments. If not, we are discussing the quality of the lock on the stable door after the horse has bolted.

OB4S4

Supporting animal welfare bodies/ethics committees with experimental design using the Experimental Design Assistant

Stringer Emma
NC3Rs, London, United Kingdom

Abstract
It is widely known that good experimental design is essential in ensuring that high quality and reproducible data is generated from *in vivo* research. This is ethically important in order to ensure that the use of animals will make a worthwhile contribution to the knowledge base. Therefore, a key role of the animal welfare body/ethics committee is to assess the quality of the experimental design as part of the project authorisation process. However, this can prove challenging as the quality and format of the information provided can vary widely.

The Experimental Design Assistant (EDA) is a web-based tool that guides the *in vivo* researcher through the design of the experiment and formulation of the analysis plan, providing automated feedback and a visual summary of the experiment. The EDA assists researchers in addressing the common problems encountered when designing and analysing animal experiments; it provides approaches that can be used to address these, and highlights where improvements are still to be made. The EDA also generates a report, which provides the key information in a format that makes it easier for members of the animal welfare body/ethics committee to determine the quality of experimental design, thereby ensuring well considered experiments, that are more likely to yield reliable and reproducible results.

OB4S5

Experimental design, robustness and the 3Rs

Jerchow Boris
Research Animal Facilities, University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany

Abstract
Research is fast, research has to be competitive and laboratory work is time-consuming, challenging and strenuous. Key competencies of scientists regularly peak where it comes to quick understanding of complex biomedical contexts and their creativity in asking new questions. The management of projects, resources, and teams is usually not part of the curriculum of scientific training and of lesser significance during a scientific career. However, the lack of project management capabilities leads to insufficient experimental design causing what we now experience as a lack robustness of published data and the reproducibility crisis, which does not seem to come to an end. We have to accept that through a better test design we achieve better results and at the same time better animal welfare. Intensive preparation and planning leads to a win-win situation. The credibility crisis of biomedical research makes it increasingly difficult to justify animal experiments. A lack of openness makes results worthless. They are questioned by the industry, they do not stand up to critical scrutiny and rightly lead to a loss of confidence in the public, which is usually already opposed to animal testing. Only meticulously planned and transparently communicated and published animal experiments make it possible to generate robust data. We need to aim for deriving the optimum benefit from animals that are as little burdened as possible. Only in this way can experiments be ethically justified and only in this way can they meet the legitimate demands of the critical public.

OB5S1

Animal research reporting update: Where are we now?

Osborne Nikki
Responsible Research in Practice, London, United Kingdom

Abstract
The goal of improving the quality of animal research reporting is one that is shared across the laboratory animal sciences. In theory it is fairly simple to achieve on a personal level once an individual is familiar with and understands how to use the free tools and resources that exist to support them to achieve this. Thus for several years a number of individuals and organisations across Europe and beyond have worked independently to improve the quality of animal research reporting. However recent studies have shown that the rate at which the overall quality of animal research reporting is improving is not as rapid as hoped[1]. This presentation will give an overview and status update for the various guidelines (including ARRIVE[2,] HARRP[3], PREPARE[4]), and other initiatives (including MERIDIAN[5] & roundtable meeting[6]) that have been undertaken to date. The factors currently limiting the rate of change will also be discussed along with some suggestions as to how through collaborative action we can all help drive culture change and increase the rate at which the quality of animal research reporting can be improved.

Dr Nikki Osborne was co-convenor of a roundtable meeting held in September 2017 to discuss how to enhance the rate at which the quality of reporting animal research can be improved. She has published several research and opinion papers relating to journal publication policies and their reporting requirements for animal studies, and was also a member of the ICLAS harmonisation of animal research reporting standards working group that published HARRP.

OB5S2

Gender bias in biomedical research

Plevkova Jana[1] and Honetschläger J.[2]
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[2]*Institute of Molecular Genetics of the ASCR, Czech Centre for Phenogenomics BIOCEV, Vestec, Czech Republic*

Abstract
The contribution discusses the problems of gender bias in biomedical research, its causes and consequences for quality of science and clinical medicine. The gender bias in biomedical research has detrimental consequences for women's health and the quality of science, because it produces bias knowledge and limits successful translation of research outcomes to the clinical practice. Uneven prevalence of several diseases e.g. multiple sclerosis that affects women 3 times more than men, partially justifies the mostly female subjects are used for experimental research in this field. However in general, female mammals have been neglected in biomedical research, because of the concern that hormonal cycles decrease the homogeneity of studied populations and therefore influence the research outcomes. Widespread prevalence of sex

differences in human diseases ultimately requires detailed experiments performed on both sexes. Even though the funding agencies encourage enrolment of female mammals, single male sex studies still predominate in the research literature. Beside the questionable quality of research performed on one sex only, there are also ethical issues concerning the female mammals produced in animal breeding facilities. FELASA should also promote and contribute to the enrolment of both sexes in biomedical research.

OB5S3

Spreading the word? The role of the veterinary surgeon in animal research reporting

Petrie Anja
Medical Research Facility, University of Aberdeen, Aberdeen, United Kingdom

Abstract
This talk does not aim to provide answers to questions. Instead it is hoped that it will be thought provoking and inspire consideration of the wider role of veterinary surgeons.

Reproducibility has become a standard agenda at CPD meetings, workshops and within institutions. People talk about a crisis; many ideas are floated on how to improve reproducibility and one concept that many agree with is better animal research reporting. The obvious group responsible for better reporting are the researchers in their publications and presenting data at scientific meetings. But how could other groups contribute to better animal research reporting? Should there be any expectations of the Designated Veterinarian (Named Veterinary Surgeon in the UK) contributing to better reporting considering that they are often at ''the coalface'' of working to improve animal welfare standards to animal research models?

As a Named Veterinary Surgeon, I feel we do have a responsibility. My talk will provide suggestions on how the vets could be involved in better reporting. I also hope that the audience will share their views either via a frank discussion or opinions collated via a turning point/ poll system.

OB6S1

How can we make research more responsible?

Osborne Nikki
Responsible Research in Practice, London, United Kingdom

Abstract
Every research student and scientist that I have ever met prides themselves on trying to be the best researcher that they can be and on conducting the best research that they can. This means that across the research community individuals are generally well motivated to conduct research responsibly. So why do we need to be discussing how to make research more responsible? The answer is that responsible research, like any other science related discipline, is a constantly evolving concept. When financial budgets are tight and individuals time is limited this can make keeping up to date with good practice a challenge in itself. This presentation will therefore discuss what responsible research currently means for those working within the laboratory animal sciences. A 'Responsible AR Checklist' will be shared, along with my own experience and reflections on how individuals priorities commonly change with respect to responsible research conduct depending upon what stage of their research career they are at.

OB6S6

Question time to funders and publishers

Riederer Beat
University of Lausanne and University Hospital, CNP, Lausanne, Switzerland

Abstract
After a short presentation from stakeholders on their perspectives on how research could be more responsible as well as an outline of selection criteria, the audience will be able to ask speakers questions regarding how responsible research is taken in account at different steps of a research project, from long term objectives of funding bodies, grant reviewers, successful funding applicants and journal publisher.

OB7S1

Ethics and sustainability in peer review

Olsson Anna S.
I3S -Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

Abstract
At a time when the academic community is heightening its attention to ethics and academic integrity, peer review seems more challenging than ever. A fair system requires peer reviewers to be able to deal honestly with the conflicts of interest which arise when asked to carry out a time sensitive evaluation of the work of competitors, and to manage the appropriate balance between ensuring critical scrutiny of the scholarly quality and being respectful towards colleagues. These issues are not new, but the rapidly growing number of published papers, the rise of predatory journals and the increased awareness of economic power imbalance in academic publishing have brought new questions into the discussion. Many feel peer review to be an unfair burden placed on them in a system where big publishers profit economically on the pro bono work of researchers. In this presentation, I will discuss these issues and present some suggestions for how editors and researchers can work together to shift peer review from painful to useful.

OB7S2

Review process and limits

Atkinson Jennie
SAGE Publications, London, United Kingdom

Abstract
At SAGE we value the work done by peer reviewers in the academic community, who provide an essential service to the process of publication excellence, driving research within their fields of expertise. The peer review process is essential to the development of research across all subject areas. There are pros and cons with the traditional model of peer review but authors and researchers benefit from having their paper improved and their knowledge developed. Reviewers also benefit from being able to read cutting edge research prior to publication and before anyone else in the field. They also have the satisfaction of knowing that they are contributing directly to the development of their chosen field. There are numerous different types of peer review, each has its own limitations but SAGE works with our publishing partners to safeguard peer review integrity. At SAGE we provide Publons to help reviewers log their reviewing activity and this also can link in with ORCID to catalogue the published and review activity of an individual. Unfortunately the peer review process is not a perfect one and it is open to manipulation. SAGE takes issues of copyright infringement, plagiarism or other breaches of best practice in publication very seriously. We seek to protect the rights of our authors and we always investigate claims of plagiarism or misuse of published articles.

OB7S3

The randomized block design and reproducibility in pre-clinical research

Festing Michael
Consultant, Burnhan Overy Staithe, Norfolk, United Kingdom

Abstract
There is a crisis in pre-clinical research. Too many experiments are giving results which turn out to be irreproducible. This is probably due to the universal use of the ''completely randomized'' (CR) experimental design. This is used in clinical trials where patients are not readily available. But it lacks power and is susceptible to bias, so is unsuitable for pre-clinical experiments where uniform animals are readily available.

Reproducibility could be substantially increased by the use of a ''Randomized block'' (RB) design. The experiment is split into a number of ''mini-experiments'', ''blocks'', or cohorts. Each block consists of a set of matched individuals, each receiving a different treatment. Each block is randomized separately. Blocks will normally be separated in time. This design is powerful, resistant to bias, operationally convenient, and reproducibility can be assessed by comparing the individual blocks.

The RB is the most widely used design in agriculture and industry. It is already being used in studies involving neonatal laboratory animals, where each litter is a ''block''. Investigators doing *in-vitro* studies also ''repeat the experiment three times'' (i.e. use three blocks separated in time) to test repeatability.

The funding organizations have an interest in ensuring that pre-clinical experiments give reproducible results. They should make the RB the default experimental design in pre-clinical research. Use of any other design would need to be specifically justified.

OB7S4

The dilemma of the editor

Riederer Beat M.
Editior-in-Chief LA; c/o Plateform of Morphology and Department of Psychiatry, Hospital of the Canton Waadt (CHUV) and University of Lausanne,, Lausanne, Switzerland

Abstract
Laboratory Animals is the international journal of laboratory animal science, technology, welfare and medicine, LA publishes **peer-reviewed** original papers and reviews on all aspects of the care and use of animals in research. Currently, only a third of all submitted manuscripts can be published and we have to be very selective. Therefore, we have to apply high standards for reporting LA research data. The decision to reject manuscripts is not taken lightly, given that for many reports animals were subject to suffering or were sacrificed. The list is extensive, with flawed experimental design, insufficient animal number, unnecessary suffering or a sex bias. Much of such mistakes could be prevented by a thorough scrutiny of research proposals and licensing review. Methodological flaws, lacking analgesia or wrong anesthesia, or lacking scoresheets and interruption criteria raise ethical concerns and make a publication impossible. When sever suffering is involved, humane endpoints are mandatory. When only one sex of animals is used, it is difficult to call it an animal model and sex bias may be considered. The review process needs to be fair and objective, and depends on responsible reporting. With increasing pressure to publish, false reporting and suspicion of scientific misconduct cannot be excluded, but such behavior is inacceptable. In conclusion, the number of submissions is increasing every year, while printing space is limited. Therefore, this puts pressure not only authors but also on granting agencies to look at the experimental design and on veterinary authorities to reinforce ethical review of research projects.

OB8S1

Genetic quality assurance and genetic monitoring of laboratory murines. A FELASA working group report

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[8]Institut für Versuchstierkunde und Zentrales Tierlaboratorium, University of Hannover, Hannover, Germany

Abstract
Genetic quality assurance (QA), including genetic monitoring (GeMo) of inbred strains and background characterization (BC) of genetically altered (GA) animal models, should be an essential component of any QA programme in laboratory animal facilities. Genetic quality control is as important for ensuring the validity of the animal model as health and microbiology monitoring are. It should be required that studies using laboratory rodents, mainly mice and rats, utilize genetically defined animals. This FELASA working group presents the objectives of and available methods for genetic QA programmes in rodent facilities. The main goals of any genetic QA programme are: (i) to verify the authenticity and uniformity of inbred stains and substrains, thus ensuring a genetically reliable colony generation-over-generation; (ii) to detect possible genetic contamination; and (iii) to precisely describe the genetic composition of GA lines.

OB8S2

Genome engineering using CRISPR/Cas9: What could go wrong?

Boroviak Katharina
Wellcome Sanger Institute, Cambridge, United Kingdom

Abstract
The CRISPR/Cas9 system is now widely established as a tool for genome engineering in many different model organisms. In mouse it is used routinely to generate models containing small insertions/deletions (indels), single nucleotide polymorphisms (SNPs), loxP sites and, less efficiently and to varying degrees of success, for the introduction of targeting constructs.

The Wellcome Sanger Institute has always been at the forefront of generating mouse models and is routinely producing mice carrying critical exon (CE) deletions and point mutations using the CRISPR/Cas9 system. There are also efforts ongoing to test and utilize new advancements within the field (such as injection method, synthetic gRNAs, . . .) to improve the efficiency of generating these mouse models.

A main part of my work focuses on generating mouse models to study human diseases. Deletions, duplications and inversions of large genomic regions covering several genes are an important class of disease causing variants in humans. Modelling these structural variants in mice required multi-step processes in ES cells, which has limited their availability. We demonstrated before that it is possible to directly generate deletions, duplications and inversions of over one million base pairs by injection of Cas9 into mouse zygotes. We followed up on this study and are now able to show that thorough analysis of these mice (for both small critical exon deletions as well as larger rearrangements) is required in order to fully understand the complexity of the alleles present within each F0 founder animal and resulting mouse line.

OB8S3

Genetic Monitoring Program: An ICLAS initiative

Perez Ana
Humodigen, Delmar, NY, United States

Abstract
Genetic monitoring is an important aspect of animal quality that is often disregarded and when overlooked, it may negatively impact scientific research. There are many aspects of genetic quality that need to be considered when establishing a genetic monitoring program in a breeding colony, especially when working with genetically modified mice or rats. ICLAS is aware of this problem and provides training sessions in several scientific venues on this topic. Additionally, it has implemented the genetic monitoring reference program for animal facilities who want to verify the genetic background of the strains they are breeding. Three internationally recognized breeders, the Central Institute of Experimental Animals (CIEA), Taconic Biosciences and Jackson Laboratories, have provided twelve of the most popular strains bred (C57BL/6NTac, C57BL/6J, NOD/LtJ, 129/SvEvTac, FVB/NJcl among others), to be used as controls. We will discuss critical steps to establish a genetic monitoring program on inbred, outbred and genetically modified mice or rats. We will provide the types of considerations needed when addressing genetic background or the genetic quality checks needed to be implemented depending on the type of technique used (pronuclear injections, CRISPR/Cas9, etc.) when establishing a mouse or rat colony.

OB9S1

Dirty or clean mice – What is better for research?

Bleich André[1] and Kränzlin B.[2]
[1]Institute for Laboratory Animal Science, Hannover Medical School, Hannover, Germany
[2]Medical Research Center, Medical Faculty Mannheim, Mannheim, Germany

Abstract
Exclusion of pathogens has been central in the standardization of laboratory rodents and remains to be important in biomedical research. However, while a century ago scientists were mainly concerned of spontaneous diseases, we are currently confronted with potential loss of phenotype after rederivation procedures as well as complaints about reproducibility or translational success. Microbiome sequencing shed light into this observation and we know that hygienic standardization came with the risk for limiting the variation of the intestinal microbiome. While the way back to undefined mice has been of great value for defined studies, large

scale application certainly raises profound ethical concerns. This session is intended to open the discussion about how to use current knowledge about the microbiome to constantly enhance the value of biomedical research in the sense of the 3Rs.

OB9S2

Are ultra-clean SPF mice too much of a good thing?

Zehn Dietmar
Technical University of Munich, Munich, Germany

Abstract
The creation of well-defined and highly controlled conditions are an essential requirement for ensuring high reproducibility of experimental findings made in laboratory mice. A key part of this standardization is the control of the hygiene status. Here significant progress had been made over the last decades and the use of tightly controlled barrier facilities, embryo-transfer requirements for importing mice into facilities, have become widely enforced standards. As a consequence, the widely held view among regulatory authorities and those involved in management of animals that increasing the exclusion of microorganisms results in better experimental conditions has been strictly enforced. However, this view underestimates how microbial exposure is part of the ''normal life style'' of higher organisms, and that the deprivation of microbial stimuli results in artificial, perturbed situations. This greatly affects not only the activity of the immune system, but far beyond. In fact, a number of significant insights were obtained over the last 5-10 years showing how pathogen pre-exposed hosts respond compared to our gold-standard laboratory mice. Moreover, we are becoming increasingly aware how microbial and microbiome derived stimuli directly, or in trans through immune cells, impact normal organ physiology.

The purpose of the presentation is to briefly summarize some of these findings and to make a strong statement that cleaner is not always better. Instead, it seems more important to improve our ability to describe the particular conditions under which data were obtained, rather than to call for uniform conditions under which experiments can only be performed.

OB9S3

Wild mice all over – Husbandry and science using wild house mice

Pfeifle Christine and Tautz D.
Department of Evolutionary Genetics, Max-Planck-Institute of Evolutionary Biology, Ploen, Germany

Abstract
Variability is an important principle of evolutionary biology and we focus our research on wild house mice (*Mus musculus*) and their variable genomes.

We caught the mice on farms and horse barns and established a wild mice husbandry in an open cage system with ten different populations of wild mice from different origins in the world.

I will describe the needs of wild mice to be considered in the daily husbandry and how to establish a balance between the demands of wild mice to their environment and good laboratory practice.

Our mouse house includes a SPF facility, gnotobiotic mice and wild mice under one roof, along with a sophisticated hygiene management. I present our 12 year experience in keeping wild house mice within a laboratory environment with the success and drawbacks we encountered. This will provide an insight into wild mice husbandry and its challenges which have to be met in respect of animal health and welfare.

Between the different populations of wild house mice and especially between wild mice and different laboratory mouse strains, we find considerable differences with respect to their microbiota and behaviour. Especially the differences in behaviour compared to laboratory strains make the husbandry of wild mice a special task. Therefore, special handling skills of caretakers and enrichment of cages are necessary and mandatory for a good culture of care.

OB9S4

Pathobionts, provocateurs, and the background microbiota – Contributions to the antigen-experienced mouse

Ericsson Aaron and Franklin C.
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Abstract
Several recent studies have demonstrated that the immune system of traditional laboratory mice is relatively immature and undeveloped, resembling that of human infants that have not yet encountered myriad antigens. In contrast, the immune systems of mice obtained from alternative sources such as pet stores or wild-caught *Mus musculus* are well-developed and resembles that of a typical human adult, raising the question of whether traditional laboratory mice are appropriate translational models in biomedical research. While pristine, barrier-raised mice and more antigen-experienced mice both clearly have utility and value, there are biosecurity considerations regarding the use of pet store and feral mice in most vivaria. Certain bacterial taxa colonizing the murine gut, including *Helicobacter* spp. and segmented filamentous bacteria (Candidatus *Savagella*) are capable of inducing host immune responses against the resident microbiota. This presentation will provide information on the experimental generation of breeder mice harboring these provocateurs, in the presence or absence of transient viral pathogens, and in the presence of different gut microbial compositions. Moreover, the downstream influence of these various microbial factors on susceptibility to two commonly used disease models will be described.

OB10S1

Modeling microbial ecosystems of mammals: current tools and challenges

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Abstract
It is now well-recognized that the microbiota plays an important role in the development, immunity and physiology of its mammalian host. Considerable progress has been made in identifying microbes and microbial metabolites that modulate host functions, maintain health or drive pathology. This knowledge has been derived from the characterization of microbiota during development and adult life, during health and disease, using animal and mechanical models to decipher the complex interactions between microbes and host, and to infer causal relationships. The 'Modeling the Mammalian-Microbiota Host Super-organism' conference, organized by Institut Pasteur and InfraFrontiers, was held on October 15th and 16th 2018 at Institut Pasteur (Paris), with the goal of bringing together specialists from fundamental and industrial backgrounds in order to discuss the state-of-the-art of research on microbiota-host/mutualism parasitism interactions, on novel methodological approaches to explore these relationships, on the diversity of animals models to study the microbiota, as well as on current challenges to access to animal models.

OB10S2

Towards understanding relevant host-microbiota-pathogen molecular interactions using the gnotobiotic Oligo-Mouse Microbiota twelve model

Brugiroux Sandrine
UMR 1071 Inserm, USC-INRA 2018, Microbes, Intestine, Inflammation and Host Susceptibility, University of Clermont Auvergne, Clermont-Ferrand, France

Abstract
Facing the tremendous complexity of conventional gut microbiota, gnotobiotic animals are powerful tools to decipher relevant molecular interactions between host, microbial populations and pathogens. Of note, gut microbiota host-specificity has been widely shown to influence both microbial colonization and enteric infection outcome, highlighting its crucial role for inter-species molecular interaction studies.

The gnotobiotic Oligo-Mouse Microbiota 12 (Oligo-MM^{12}) model harbors twelve bacterial strains all isolated from conventional mouse microbiota and assigned to five major bacterial phyla: Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria and Verrucomicrobia. Interestingly, intestinal colonisation of Oligo-MM^{12} consortium was stable over time and mouse generations, which represents an important requirement for the establishment of isobiotic mouse lines. Stable Oligo-MM^{12} colonisation was also observed accross mouse facilities, strengthening experimental reproducibility and conclusions. Finally, the open accessibility to each Oligo-MM strains, genomic sequences and specific analytical methods such as 16SrRNA gene-based assays, together combined with meta-omics approachs favors mechanistic studies.

Thus, since its establishment, the gnotobiotic Oligo-MM^{12} model has been a major asset to decipher molecular interactions between microbial members and human enteric pathogens such as *Salmonella enterica* serovar Typhimurium and *Clostridium difficile*. Undoubtedly, this model will be of great help in other studies.

OB10S3

Animal source and husbandry: Implications for model phenotypes in biomedical research

Ericsson Aaron and Franklin C.
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Abstract
Recent advances in culture-independent methods to characterize polymicrobial communities such as the gut microbiota (GM) have revealed an ever-growing number of associations between characteristics of the GM and a wide range of human health conditions, both physical and mental. Similarly, a growing body of evidence suggests that differences in the GM of animal models may contribute to poor reproducibility of pre-clinical research using animal models, and poor translatability to the human conditions they are intended to model. This presentation will provide data generated in multiple model species showing the influence of several underappreciated factors capable of influencing the composition of the GM, including animal source, transportation, acclimation, caging, bedding, and water treatment. Examples will also be given of changes in the GM of animal models induced by these factors and the resulting changes in model phenotype. Lastly, recommendations will be provided regarding methods to manipulate the GM experimentally and monitor colonies long-term for changes in the GM due to institution-specific factors.

OB10S4

Microbiota composition influences the colitogenic effect of murine norovirus

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Abstract
Changes in bacterial composition of the gut microbiota have been associated with the development of diverse human disorders such as inflammatory bowel disease (IBD). Although exact mechanisms

of IBD are not fully understood, its onset includes interplay of multiple factors including genetic predisposition, environmental factors and intestinal microbiota. The functional and causal microbe-host interactions are difficult to address due to high microbiome complexity. Therefore, we used a gnotobiotic mouse model, in which experimental IBD was reduced to three factors: susceptible host, defined bacterial consortia, and murine norovirus (MNV) as the disease trigger. The aim of this study was to determine how specific composition of minimal microbiota influences the outcome of MNV triggered colitis in an interleukin-10 deficient ($IL10^{-/-}$) mouse model.

$IL10^{-/-}$ mice colonized with Oligo Mouse Microbiota (OMM) developed mild inflammatory lesions in the colon, whereas in mice associated with Altered Schaedler Flora (ASF) only few animals showed histopathological changes. MNV infection triggered moderate colitis in mice carrying ASF characterized by epithelial hyperplasia and inflammatory cell infiltration, but not OMM colonized mice. Interestingly, co-colonization with segmented filamentous bacteria (SFB) abolished intestinal inflammation only in mice carrying ASF. SFB presence in ASF gnotobiotic mice after MNV infection induced upregulated expression of barrier determining factors and triggered pro-inflammatory and regulatory immune response in the colon.

In conclusion, the colitogenic effect of MNV depends on the presence of specific bacterial species. Furthermore, the co-colonization of SFB prevents MNV triggered colitis by boosting the epithelial barrier function and activating the host immune system.

OB11S1

Impact of technological evolutions on global operational management and sterilization issues

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Abstract
Gnotobiology and microbiota-related studies play an increasing and extending role in understanding multiple interactions with mammalian hosts, in many fields such as immunology, degenerative diseases, ageing, neurosciences... Conducting gnotobiology studies requiring the comparison of different microbiota is very often problematic or impossible in isolators, due to various constraints which include experimental design and procedure limitations, impossibility to maintain multiple gnotoxenic groups separately, room capacity and cost limitations, etc. The presentation aims at reviewing some selected evolutions, sharing experience in addressing these challenges through technical solutions allowing an improvement of experimental design and operational management of studies. It will include bioexclusion systems available for breeding, housing and experimental procedures according to the type study and the microbiota definition, and solutions in cold sterilization.

OB11S2

Accessing gnotobiotic mouse models: example of the centre for gnotobiology the Institut Pasteur

Berard Marion, Maranghi E., Angélique T., Jacob M., Diakhate H., Ferrandez S., Maucourant V., Orumcek A. and Vimont E.
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Abstract
Homeostasis between microbiota and the host ensures vital functions of the organism. Disruption of this homeostasis can lead to severe illnesses of major impact on public health.

Understanding the interactions between microbiota and the host is the basis of numerous large projects in Europe and beyond. Essential tools for this type of research are germfree (GF) and gnotoxenic (GX) mice.

In this context, the Centre for Gnotobiology of the Institut Pasteur plays an essential role for numerous research groups for which they breed, quality control, distribute GF mice and implement projects on GF and GX animals.

We will present the different steps of development of this platform and our current operations, focusing on the breeding and the implementation of protocols.

OB11S3

Microbiota implantation and monitoring: new challenges, ongoing innovations and evolutions

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Abstract
Due to the increasing amount of research works on the effect of the commensal microbiota in health and disease, the use of germ-free animal models is expanding. Germ-free animals are animals devoid of living microorganisms. They are mostly compared with conventional animals, in order to assess the importance of commensal microbiota in host physiology regulation. Before undertaking any experiment with those models, it is mandatory to ascertain their germ-free status. Microbial cultures, microbial cell viability tests, and molecular methods based on microbial DNA extraction and amplification are currently in use. Procedures used in the Anaxem facility of the Micalis Institute will be presented, and advantages and disadvantages of the different methods will be discussed. Germ-free animals are also often used as hosts of various kinds of microbiota, from a particular entity to a complex ecosystem. Indeed, transplanting the commensal microbiota from a donor organism with a phenotype of interest (such as a disease) to a naive recipient organism, allows demonstrating the causal role of the microbiota in the phenotype. Yet, microbiota transplantations raise methodological issues, including the preparation and conservation of the microbiota transplant, the use of a microbiota

transplant prepared from a single donor or from a pool of donors, the transplantation method, the age of colonisation, the time required to reach an equilibrium between the recipient organism and the microbiota transplant. So far, there is no consensus on the best methodology. Several examples will be presented and the advantages and disadvantages of the different strategies will be discussed.

OB12W1

Making experimental design education more effective

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Abstract
A widespread need for improving experimental design is indicated by the considerable literature questioning the quality of published research. Clearly this is a matter that should be addressed in the ''adequate education and training'' required under Directive EU2010/63 for scientists who design procedures and projects. This session should help course providers exchange ideas on how to make researchers aware of the risk of poor study quality and of possible improvements in how they design and conduct experiments.

It will explain the key concepts the presenters have thought important to convey in education and training courses covering experimental design, and why. It will also show some of the techniques that have been used to make experimental design tuition interesting and informative, and lead on to discussion on how these and other approaches might help improve the learning experience and knowledge transfer for this topic.

As follow-up to the session, those involved in educating scientists using laboratory animals could meet to consider the idea of an international experimental design working group and discuss ways to increase the number of tutors able to educate others in the topic. This could help provide a consistent approach across the international laboratory animal science community.

OB13S1

Can criterion based interviews validate competence in designing animal experiments?

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Abstract
According to the Directive 2010/63/EU, we have to ensure competence levels of personnel involved in animal experiments including those ''designing procedures and projects''. The importance of such competences is emphasized by observations that most research findings within animal research are questionable due to poor methodology. In response to these claims, new standards in the design of animal experiments have been introduced. Adhering to them requires thorough knowledge of the model, statistics and animals, and can be very specific for different types of experiments. In line with these growing insights, it is important to establish how well researchers are able to comply with the guidelines and are able to use them to improve the quality of their experimental design. By Validation of Prior Learning one could establish the level of competence. It should be considered that assessment methods should be available independent of training and as an aspect of lifelong learning in animal science. We would like to debate the potential of self-assessment methods followed by criterion-based interviews (CBI) to establish competence. This could also reveal specific educational needs, subsequently offered. We will propose to implement such an assessment as part of protocol reviewing, assessing not only the quality of the protocol but also the competence of the applicant. The structure, risks and values of such tools will be discussed. We will present the advantages and possibilities of CBI as an assessment tool, and the requirements it imposes on the competence of appointed assessors.

OB13S2

Round table: Train the trainers on experimental design

Mocho Jean-Philippe
FELASA

Abstract
Following the issues highlighted in the previous session and presentation, this round table is the opportunity to brain storm with LAS industry stakeholders on how to make experimental design education more effective. To start the discussion, the results of a pan-European survey on teaching of experimental design for in vivo research will be presented.

OB14W1

How to recognise sloppy science

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Abstract
Unfortunately, there is no metric for scientific quality. Rather, the journal in which a paper is published, and number of citations are both used as surrogates for ''quality''.

The purpose of this session is to identify the common features of ''sloppy science''.

In this workshop we will review several de-identified, highly cited publications, from famous investigators published in ''top tier'' journals.

We will dissect them together to illustrate the systemic problems that are evident throughout the literature. Those deficiencies typically include lack of investigator blinding, lack of positive

and negative controls, ''cherry picking'' the data, failure to repeat experiments, use of non-validated reagents, and inappropriate analyses of the data. These characteristics are common to papers that represent poor-quality, non-reproducible science.

OB15S1

The serendipitous discovery of a novel murine astrovirus (MuAstV2) with unusual properties

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Abstract
Soiled bedding exposed sentinel mice from multiple adjacent holding rooms in a 4-room vivarium seroconverted to MTLV on a multiplex immunofluorescence assay (MFIA) using a novel antigen produced in an AKR T-cell line. The mice were seronegative using MTLV antigens obtained from MTLV-infected neonatal thymocytes. The sera from these mice also reacted with the uninfected AKR T cell line in an immunofluorescence assay, and the cell line was MTLV-positive by mouse antibody production test using the aforementioned MFIA. Using metagenomics, a novel murine astrovirus, which we have designated murine astrovirus 2 (MuAst-2), was identified in feces from Swiss Webster mice placed as dirty bedding sentinels. MuAst-2 is closely related to viruses isolated from feral rats in China and feral mice in NYC, and is genetically distinct from the prevalent MuAst-1 found in many contemporary mouse colonies. Further investigation revealed that highly immunodeficient NOD.Cg-Prkdc^{scid}IL2rg^{tm1Wjl}/SzJ(NSG) mice do not become infected when co-housed with MuAst-2 shedding SW mice; using qRT-PCR, C57BL/6NCrl(B6) mice orally infected with MuAstV-2 shed virus beginning on Day 2 post-inoculation, peaking on Day 5, with shedding ceasing between Days 56-168, whereas immunodeficientNOD-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}/NjuCrl(NCG) mice do not; and, viral RNA particles were detected in the intestinal epithelium (enterocytes and/or mucosal immune cells) using MuAst-2 specific *in situ* hybridization. In addition, cells within the lamina propria, primary and secondary lymphoid organs, as well as intravascular leukocytes showed evidence of MuAstV-2 RNA, suggesting replication in these sites as well.

OB15S2

Detection methods for Murine Astrovirus in individually ventilated caging systems

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Abstract
Introduction: Murine Astrovirus (MuAstV) is a common viral pathogen in laboratory mice with high prevalence in research and commercial breeder colonies worldwide. Although infections do not cause clinical signs it must be assumed that at least the immune system may be affected. A study was conducted to investigate suitable strategies for reliable detection of MuAstV in individually ventilated cages (IVC).
Methods: A defined number of cages with MuAstV infected mice was kept in an IVC rack together with an otherwise MuAstV negative colony. Results obtained by soiled bedding sentinel (SBS) fecal PCR and serology were compared with those gathered by exhaust air particle PCR from the air handling unit of the IVC. The experiment was repeated seven times.
Results: In 5/7 repetitions, sentinels became infected with MuAstV through dirty bedding as demonstrated by serology. In 6/7 repetitions, SBS fecal PCR was positive, whereas EAP-PCR detected the virus in each repetition (7/7).
Conclusion: MuAstV can be detected at low prevalence by both methods, exhaust air particle PCR and soiled bedding sentinels. Since the virus was not detected in each repetition by SBS serology and fecal PCR, these methods harbor the risk of missing infections. Testing exhaust air samples by PCR represents a better alternative due to accumulation of MuAstV-RNA in the exhaust air dust.

OB15S3

Mouse Kidney Parvovirus: A newly characterized parvoviral pathogen of research mice

Besch-Williford Cindy, Crim M., Hart M. and Livingston R.
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Abstract
For decades, a nephropathy of unknown etiology caused tubular epithelial intranuclear inclusions in kidneys of immunocompetent and immunodeficient mice. Recently, the etiology of this condition was identified as a parvovirus of the chapparvovirus genus. Divergent genetically from the murine parvoviruses MVM and MPV, this new virus was named Mouse Kidney Parvovirus (MKPV). MKPV replicates in the kidney, and is detected in blood, kidney, and urine of infected mice. Due to voiding habits of mice, MKPV is also found in feces collected from the cage. Infection in immunocompromised mice is persistent, causing progressive renal damage, while infection in immunocompetent mice appears to be transient once an antibody response is generated. Virus can be transmitted horizontally by housing naïve mice on virus-contaminated soiled

bedding or with infected mice. Viral transmission can also occur by passage of MKPV-contaminated xenografts and biological materials. Diagnostic tests include PCR of kidney, feces collected from soiled bedding, environmental samples (cage swabs and rack exhaust filters) or biological materials. Virus-infected research mice have been found globally and the prevalence of MKPV, as tested by PCR from samples received in our laboratory, is about 12%. Inclusion of MKPV in health monitoring and biologic material testing is recommended to prevent use of infected mice or mouse-passaged biologics in research investigations.

OB15S4

Persistence of Murine Parvoviruses after embryo-transfer: Addressing the problem via a novel qPCR assay

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Abstract
Embryo transfer (ET) is a standard procedure to obtain specified-pathogen-free laboratory animals. However, we have observed a residual risk of parvovirus transmission during ET, which in combination with strain dependency of seroconversion necessitated direct virus detection in addition to serology. In this study we designed a high-sensitive quantitative polymerase chain reaction (qPCR) assay and utilized it to assess parvovirus transmission during rederivation of a parvovirus-positive mouse strain. ET was performed using CD2F1 foster dams, which were sacrificed for hygienic monitoring after weaning of their litter, including serological analyses by indirect immunofluorescence-assay (IFA) and qPCR-testing of necropsy and environmental sample material. Mice from F1- and F2-generations of the re-derived mouse strain were screened for parvovirus transmission by qPCR of environmental sample material. Sampled organs from positive tested animals were homogenized and administered to parvovirus-negative recipient mice, aiming at differentiating infectious virus from residual DNA. ET of embryos from infected donor mice led to seronegative foster dams, whereas virus was detected in lymph-nodes, spleens and environmental samples, showing that animals became carriers without seroconverting. qPCR based screening of the offsprings revealed virus-DNA in various sample materials, whereas specific antibodies were only found in one out of 33 tested animals, indicating virus persistence in following generations. Provoked virus transmission by injection of positive tested organs into a recipient cohort resulted in enlarged lymph-nodes and positive qPCR results, pointing out infectiousness of detected virus material. These results underline that monitoring parvovirus transmission after ET requires multifaceted sampling that includes high-sensitive direct virus detection.

OB15S5

Zebrafish Picornavirus: Surveillance for a novel viral pathogen of laboratory zebrafish

Crim M., Besch-Williford Cindy, Hart M. and Livingston R.
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Abstract
Subclinical infections of research animals can add confounding variability in animal studies and have the potential to alter experimental outcomes. Zebrafish picornavirus 1 (ZfPV-1) is a recently discovered novel picornavirus that subclinically infects laboratory zebrafish. ZfPV-1 replicates in the enteric mucosa and transmission is presumed to be fecal-oral, which is a common mode of transmission among picornaviruses. While the virus can be detected in clinically normal zebrafish, the impact of infection on the zebrafish immune system, gut development, microbiome, and other research areas remain unknown. Health monitoring data from zebrafish colonies indicate that the virus is prevalent, with 24% of samples testing positive to date, and widely distributed among research institutions in Europe and North America. ZfPV-1 can be detected by real-time PCR in a wide variety of sample types, including environmental samples, feces, embryos (presumably reflecting fecal contamination), and whole frozen zebrafish. Fecal samples, which are sensitive for viral detection, can be easily collected from small groups of adult zebrafish and are useful as an antemortem test for valuable zebrafish lines or fish held in quarantine. To prevent potential adverse impacts on research from an unrecognized viral infection, inclusion of ZfPV-1 is recommended for colony health monitoring and quarantine testing.

OC1S1

The advanced methods for preclinical imaging of small laboratory animals

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Abstract
The anatomical and molecular imaging methods for in vivo imaging of small laboratory animals are subject of rapid development during the last decade. The new methods like magnetic particle imaging (MPI) and whole body photoacoustic imaging (PA) complemented the established imaging methods. While MPI allows high sensitivity/high speed quantitative nonradioactive molecular imaging, PA brings the molecular imaging resolution one order of magnitude down compared to other molecular imaging methods. The X-ray/CT imaging belongs to fundamental anatomical imaging methods. Nevertheless, it suffers with poor soft tissue resolution and high radiation dose delivered during CT examination. The new photon-counting radiation detectors allow significant improvement in tissue discrimination (spectral or color CT), increasing imaging speed and diminishing acquired radiation dose. The same

detectors could be used in positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging. We present a proof-of concept of the feasibility of Timepix 3 detectors for CT, PET, SPECT and XRF (X-ray fluorescence) whole body imaging. The simultaneous recordings of the time of flight, position, and energy of interacting photons allow to filter unwanted events and increase the sensitivity, signal-to-noise ratio, and speed of imaging. Construction of Compton camera enables the collimator-free SPECT imaging with significantly increased sensitivity which allows to reduce amount of necessary radiotracer and thus diminish the harmful radiation exposition of investigated object.

OC1S2

Interferon-beta Reporter Mice: An in vivo imaging tool for non-invasive Toll-Like Receptor stimulation studies

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Abstract
Toll-like receptor (TLR) activation is a fundamental part of innate immune mechanisms and therefore plays a crucial role in pathogen defense, inflammatory diseases as well as autoimmune disorders. CD14 is a well-established co-receptor for TLRs, however little is known about its impact on tuning pro-inflammatory and regulatory cytokine expression. Kinetics of induction and downregulation is crucial in this context which is why traditional tissue based readouts often require the use of larger animal cohort's to assess different measurement time points. We previously identified CD14 as a modifier of colitis and as Type-I interferon was shown to be protective in colitis models we aimed to investigate the CD14 dependent Interferon-beta (IFN-β) induction after TLR triggering.

In the present study we established an *in vivo* imaging tool to non-invasively assess the impact of CD14 on TLR induced IFN-β expression. We generated a *Cd14* deficient IFN-β reporter mouse line and compared them to respective ''wildtype'' reporter mice. Mice were treated with different TLR-ligands and IFN-β induction was noninvasively measured using an ''In Vivo Imaging System'' (IVIS). The *findings* obtained by IVIS were validated by traditional detection methods. As expected, all mice treated with TLR ligands revealed marked IFN-β expression when compared to untreated mice. Importantly only LPS dependent IFN-β induction was impacted by CD14 deficiency. During the present study *Cd14*-deficient IFN-β-Reporter mice were proven to be a valuable *in vivo* tool analyzing TLR induced IFN-β expression. Thus this model offers the opportunity for complex longitudinal studies, substantially contributing to the 3R's principle.

OC1S3

Choice of anesthesia is critical for revealing glucometabolic changes by functional brain imaging

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Abstract
Preclinical positron emission tomography (PET) imaging is a powerful tool for longitudinal in vivo measurements, thereby reducing animal numbers. For preclinical PET, anesthesia is usually unavoidable but also influences the distribution of radiotracers like 2-[^{18}F]-fluoro-deoxy-D-glucose (^{18}F-FDG) used for imaging of glucose metabolism. For reliable data interpretation, structured analysis of anesthesia impact on basal glucose metabolism and disease-associated changes is needed.

Here, we used naïve rats (n = 7–11 per anesthesia) and rats during epilepsy development (n = 4–12 per timepoint and anesthesia) known to exhibit cerebral glucometabolic changes to compare four anesthesia protocols: ^{18}F-FDG uptake phase in conscious rats followed by a static brain PET scan, and dynamic scans (allowing for pharmacokinetic modeling) under either continuous isoflurane, medetomidine-midazolam-fentanyl (MMF), or propofol anesthesia.

In naive rats, isoflurane and propofol anesthesia resulted in significantly decreased cortical ^{18}F-FDG uptake while MMF anesthesia led to a globally decreased brain uptake compared to uptake in conscious rats. During epileptogenesis, MMF anesthesia was best distinctive for visualization of prominently increased glucometabolism in epilepsy-related brain areas. Kinetic modeling further increased sensitivity, particularly for continuous isoflurane anesthesia. During chronic epilepsy, hypometabolism affecting almost the whole brain was detectable with all protocols.

This study exemplifies that careful selection of anesthesia protocols for functional imaging can prevent the generation of false negative data. Importantly, evaluation of anesthesia protocols should not only include healthy controls but also animals of the disease model of interest.

OC1S4

In vivo imaging of tumor cell migration and metastasis

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Abstract
Although much knowledge has been gained about the onset, progression and treatment of cancer, it is still one of the leading causes of death in the Western world. Cancer-related death is mainly due to metastasis: the ability of tumor cells to colonize

distant sites after detaching from the primary tumor, migrating and traveling through the body via the blood circulation. Understanding the mechanisms underlying the development of tumors and the metastatic process is vital to design and develop drugs that specifically target (the formation of) metastases and prevent further metastatic outgrowth.

To study the dynamic processes involved in cancer, we have developed various imaging windows to image e.g. intestinal, liver, brain and breast tissue, and visualize the behavior of individual cells at subcellular resolution in living mice with two-photon intravital microscopy (IVM). Using this state-of-the-art imaging technique, the rare but dangerous population of tumor cells that has the ability to detach from the primary tumor and form distant metastases can be investigated. By combining these imaging techniques with genetically engineered mouse (tumor) models, the rare but dangerous population of tumor cells that has the ability to detach from the primary tumor and form distant metastases can be investigated. This provides our lab unique opportunities to study dynamic processes including epithelial to mesenchymal transition (EMT), stem cell capacity, cell-cell communication and response to chemotherapy of tumor cells.

OC1S5

Using 3D imaging and AI to improve scientific and 3Rs outcome in cancer research

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[2]*Marketing, Fuel3D, Oxford, United Kingdom*

Abstract
In oncological drug development, animal studies continue to play a central role, with the volume of subcutaneous tumours closely monitored to assess the efficacy of new drugs. The standard measurement technique of handheld callipers results in subjectivity, a lack of traceability and errors in volume accuracy, resulting in repeat studies, more mice being used and longer, more costly trials. We demonstrate the extent of inconsistencies from a dataset of 2,500 tumour calliper measurements derived from 1,600 mice (6 strains), multiple operators and 20 tumour models. We will show how an innovative new solution combining 3D stereo photometry, thermography and machine learning enables tumour measurements to be recorded in a rapid, non-invasive, morphology-independent way, reducing human-bias while providing full traceability for studies and greater measurement accuracy for irregular tumours.

Further, we examine how our multimodal imaging platform can with the help of machine learning automatically extract biomarkers (such as tumour condition, i.e. ulceration, redness, pallor, necrosis) for animal welfare and toxicity/efficacy endpoints. To train our classifiers, scans are reviewed and labelled by industry experts to ensure robustness. The classifiers are then applied onto unseen scans to rate their tumour condition. This new tumour classification tool in development a 3Rs organisation will enable animal technicians and scientists to make more confident and informed animal welfare decisions.

A further benefit of digitalising tumour measurement is the creation of a non-subjective standard methodology, opening the door for cross-trial and industry collaboration to advance oncological research.

OC2S1

Importance of the olphactory environment in welfare of the laboratory animals

Fekete Sándor György
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Abstract
The 3rd R, the refinement is receiving more and more attention. The optimazing of the auditory and olphactory environment of the laboratory animals is essential, because the environmental smells influence the behaviour and occasionally the epigenetics of mammals (Korsós et al. 2018). The intraspecific communication is attained by means of the pheromones, especially in the social and sexual relations. The HEPA-filter of rodents' cages does not isolate smells. Sebestény et al. (2011) reported about an outbreak of Pateurella-caused prostatitis in rats, under the influence of female pheromones. In rat experiment the smell substance of the fox faeces (2,4,5-trimethyl-tiol=TMT) caused an expressed freezing reaction. In the experiment of Korsós et al. (2017), the effects of neutral and irritative smells and the synthetic trimethyl-tiol (TMT) upon the open-field (OF) behaviour of rats, with the background pathophysiology and histology were studied. The passive ethological elements were overwhelming under the influence of the concentrated TMT and MHA. The grooming was generally rare. Summerizing, the diluted synthetic TMT partly brings about the effect of fox faeces, while the concentrated TMT is a repellent. The citronella represents a light stressor. Freezing was detected rarely and the final corticosteron values did not differ from the physiological ones and both necropsy and histopatology revealed no pathological alterations. Taken together, the short-term smell effects are not able to develop chronic stress. Further studies required to elucitate the long-term effects of smells and to differentiate between irritative or species-related effects of smells and to formulate recommendations for the practice.

OC2S2

Effects of spraying gloves with alcohol on mouse behaviour before, during and after handling

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Abstract
Alcohols are routinely used in laboratory animal facilities to disinfect items such as hands, surfaces and equipment. We hypothesized that if mice perceived alcohol as aversive they would display

increased behavioural welfare indicators relevant to fear/defence (e.g. defensive burying, rearing), approach/avoidance (interaction with the hand), and social interactions (e.g. aggression). We observed C57BL/6J and Balb/c mice before and after handling them with nitrile gloves sprayed with 70% Ethanol or not sprayed with anything. Additionally, we assessed how these handling methods affected home cage behaviour. Both strains displayed increased wall rearing, and C57BL/6J mice showed defensive burying, during the anticipation of handling when alcohol was present, suggesting that mice perceived this scent as threatening or aversive. After handling with alcohol, mice increased self and allogrooming and, depending on the strain, reduced the frequency they approached the hand and increased wall rearing, possible indicators of avoidance. When the cage was returned to the rack, handling with alcohol decreased aggression in males in favour of self and allogrooming. We can conclude that, in the presence of alcohol, behavioural indicators such as wall rearing, defensive burying and avoidance of the hand indicated that handling mice with this hand sanitizer might affect mouse welfare negatively. However, the potentially positive effect was that, in males, it reduced home-cage aggression for the 20-minute observation period, possibly replaced by allogrooming and sniffing, although it is not known if aggression was decreased or merely delayed.

OC2S3

Strains dependent behavioural responses of female mice to urine of antigen-treated males

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Abstract
The important component in preventing the spread of infection in populations are the detection and avoidance of infected partners. Disease information is often displayed by the infected animal and include the changing in the odor and in scent-marking activity. The decrease in the odor attractiveness of infected animals is largely due to the immune reactions and changing in the ratio of pro- and anti-inflammatory cytokines. We studied the intra-strain behavioral responses of females to urine samples of healthy and antigen-stimulated males in strains differing by their predominant Th1 (C57Bl/6) or Th2 (BALB/c) immune response. Analysis of the behavior in the olfactory test showed that the BALB/c females spent more time sniffing urine samples than the C57BL/c females ($F_{1,103} = 126.4$, $p < 0.001$). C57BL/6 females exhibited more digging activities ($F_{1,103} = 13.7$, $p < 0.001$). The grooming time was the same for both strains ($F_{1,103} = 0.01$, $p = 0.97$). The effect of antigen stimulation by KLH to odor of urine was found only for sniffing time ($F_{1,103} = 4.9$, $p = 0.03$). The BALB/c females spent more time sniffing urine from control males compared to samples collected 3 days after antigen stimulation, whereas the C57BL/6 females did not discriminate urine of healthy and antigen-stimulated males. These differences could be determined by the type of immune response. The higher sniffing activity of BALB/c (Th2) females may contribute to the choice for healthy mating partners compared to C57Bl/6 (Th1) females.

OC2S4

Effects of cage density and sanitization frequency on animal

Naden Jamie
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Abstract
The 8th edition of the Guide for the Care and Use for Laboratory Animals (*Guide*) has recommendations for the minimum amount of space required for rodents that are housed in groups as well as cage sanitation frequency. Adjustments to these guidelines may be allowed if reviewed and approved by the institution's IACUC and any changes should be based on performance indices of animal well-being and research quality. The purpose of this study was to evaluate the effects of cage density and sanitization on weight gain, animal welfare and respiratory lesions that could be associated with ammonia concentration in rodent cages. The *Guide* recommendations were compared to the standards adopted at Envigo rodent production barriers. The study was completed using Hsd:Sprague Dawley® SD® (SD) rats and C57BL/6NHsd (B6) mice. Breeding cages were housed according to internal standards and *Guide* recommendations. The number of pups born and weaned was recorded, as well as pup and adult morbidity, mortality, cage ammonia, clinical conditions, animal behavior, and cage cleanliness. After weaning, pups were housed according to *Guide* and internal standards. Pup body weights was recorded from weaning through 12 weeks of age. At the end of the study, five animals per group were randomly selected from different cages and were examined for pathology of the nasal cavity and lungs. Overall, we found that internal standards are comparable to the *Guide's* recommendation with no detrimental effects of using the higher cage density and lower sanitization frequency.

OC2S5

Comparison of laboratory rat behaviour and welfare in two different cage housing conditions

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Abstract
The design of any captive animal housing system should aim to promote welfare by encouraging species-specific behaviours, however, many laboratory animal cages fail to meet the needs of the animals, providing either insufficient space and/or complexity to allow the expression of full behavioural repertoire. Over a ten

week period we studied 12 groups of four twelve-week old male Sprague-Dawley rats housed in either 'standard' laboratory rat cages (58cmL, 38cmW, 22cmH) containing a shelter, tunnel, nesting material and aspen chew block, or larger ("Tower") cages (73cmL, 58cmW, 46cmH) with multiple shelters, perches, nesting material and locations, tunnels and the aspen chew block. Behavioural data was collected from video during periods of relatively high activity in dark phase, with activity, posture and location compared between the two housing systems. Rats in Tower cages groomed less ($F_{1,50}$= 55.80, p = <0.001) and were more likely to show tactile social behaviours (t = 2.60, p = 0.026) such as placing a paw on another rat. Rats in Tower cages performed fewer antagonistic behaviours such as; prolonged pin-down of a subordinate rat, without allowing escape, (t = −2.86, p = 0.017), and fewer bouts of sleep ($F_{1,50}$ = 27.83, p = <0.001) than those in standard cages. The increase in positive affiliative behaviour, reduced aggression and reduced self-directed behaviour suggests that rats housed in Tower cages have improved welfare compared to rats in Standard. All animal studies were ethically reviewed and carried out in accordance with the GSK Policy on the Care, Welfare and Treatment of Animals.

OC2S6

Using epidemiology to approach aggression in group housed mice

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Abstract
Aggression between male mice is one of the most common problems in laboratory mouse husbandry. Group housing is fundamental to ensure good animal welfare, social support from conspecifics also markedly improves health outcomes, and therefore model quality. Refining present husbandry routines and find strategies to prevent the problem is thus crucial. We used an epidemiological approach to collect data from existing populations, a method previously proven powerful and non-invasive to address important welfare concerns and increase the understanding of environmental and biological factors affecting multifactorial problems. Data was collected over a period of one year across Stanford University. Racks in rooms were randomly selected and cages visually assessed one at a time to collect data on fighting and related trauma. Independent variables included factors such as time of year, cage type, enrichment, rack position, sex, strain etc. Data was analyzed using nominal logistic regression and generalized linear modeling. In total, fighting was noted in 13.8 % of male mice housed in groups of two or more, with 13.8 % of these having signs of trauma. Results revealed increased levels of aggression in mice housed on the top racks, and in individually ventilated cages with corncob bedding. Other predictors of aggression included strain and time of year. This indicates that aggression is a complex behaviour, with several factors contributing to determine the severity of the problem. During the spring a pilot study is conducted using the same protocol to collect data in Swedish mouse facilities, results from this will also be presented.

OC3S1

Best practices for noise and vibration in the vivarium

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Abstract
Noise and vibration are present in every laboratory animal vivarium room, with great variability from room-to-room and facility-to-facility. Such stimuli are rarely measured. As a result, the many stakeholders (e.g., funding agencies, oversight bodies, construction personnel, equipment manufacturers, animal facility administrators, veterinarians, technicians, and scientists) have little awareness of the relative impacts such stimuli might be having on their animal models. This presents an uncontrolled source of variability in our vivarium space, a potential source of unrecognized animal distress, and a potentially major uncontrolled confounding variable in our scientific studies. Unmeasured and unrecognized noise and vibration can therefore serve to limit our 3R's goals of refining our animal models and reducing the number of animals used in biomedical and behavioral research. This presentation will highlight the scope of the noise and vibration problem, when and how to measure them, what levels to avoid, and the importance of having a written noise and vibration training, communication, and action plan.

OC3S2

Is laboratory animal husbandry adequately reported in published articles?

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Abstract
The ARRIVE guidelines are aimed at improving the reporting of research studies that utilise animals and minimise unnecessary repetition of studies. The guidelines recommend that researchers should provide details of husbandry conditions and details of animals used in experiments. However, despite the recommendations there is variation of reporting in published articles.

The current study sought to do a literature search and compare different husbandry conditions reported in published articles. Common methods for data retrieval were used, which were to search the PubMed database for articles published in the year 2017. The words "rat model + animal study" were searched to retrieve the articles and the following filters to refine the search; article type: clinical trial, clinical study, comparative study and text availability: free full text. The methodology section of articles that were free full texts and published in English was

reviewed and information about acclimatisation, age, sex and housing recorded into an Excel spreadsheet.

A total of 100 articles was randomly surveyed and most of them reported the species (98%) and animal sex (89%) used while not reporting the type of food (79%); acclimatisation period (80%), housing temperature (70%); humidity (87%) and light intensity (99%). The results from this study are a cause of concern as they provide evidence that there are inconsistences in the reporting of husbandry conditions. inconsistent reporting has a major impact on reproducibility of studies and we therefore recommend that journals should ensure that ARRIVE guidelines are implemented whenever possible.

OC3S3

Do different caging systems affect the outcome of behavioral tests in C57BL/6J male mice?

Moltsanidou Eleni[1], Polissidis A.[1], Dalla C.[2], Balafas E.[1], Alexakos P.[1] and Kostomitsopoulos N.[1]

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Abstract

Studies have shown that housing can affect the health and well-being of laboratory mice. Consequently, housing may affect the outcome and the reliability of the experimental results. The purpose of this study was to assess the role of the caging system in the exploratory and anxiety-related behavior of mice. Three widely known housing systems, Open Top Cages (OTC), Motor Free Ventilated Cages (MFVC) and Individually Ventilated Cages (IVC), were used. Subjects were 25 days-old male C57BL/6J mice (n = 36), equally assigned to the three different caging systems, MFVC, OTC and IVC. The experimental process consisted of three behavioral tests: the open field test, the elevated plus maze test, and the three chamber sociability and social novelty test. Results from the open field and the elevated plus maze test revealed that mice housed in the MFVCs showed increased exploratory and less anxiety-like behavior. The three chamber sociability and social novelty test showed that mice of all three caging systems equally preferred to spend more time with another mouse (sociability) and to investigate a novel mouse more than a familiar one (social novelty). It is concluded that different caging systems may influence the exploratory and anxiety-like behavior of laboratory mice. It is essential to take into account the housing conditions when designing and performing experimental protocols as well as when reporting, analyzing, and systematically reviewing the results of behavioral testing in mice.

OC3S4

Only the lonely and what to do about it

Florijn Wouter

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Abstract

Prolonged social isolation (SI) of mice is regularly practiced following bite wounds caused by male fighting. Increasingly, SI is used as a solution to prevent male aggression and its subsequent effect on experimental read-out parameters. Due to the wide spread use of individually ventilated cages, no visual, auditory, olfactory and/or tactile contact will be maintained, which may be considered as a violation to the European Directive (3.3a, Housing and enrichment) and should be classified as severe suffering (section III,3k).

Confronting researchers with these statements leaves them flabbergasted as they observe docile,

well fed individually housed mice without any visible behavioral aberrations. Lack of knowledge of the overwhelming amount of literature/reviews showing a broad range of

striking differences in behavioral and physiological read-out parameters between group or

individually housed mice and rats appears to be the case. Although observers detect no visible signs of discomfort, research shows several effects following a few weeks of solitary housing.

We will discuss alternatives for individual housing, provide information on the use of a buddy and will show preliminary data on the use of a hormonal implant in female companion mice.

Subcutaneous injection of a tiny portion of suprelorin implant prevents pregnancy in female mice for many months.

OC3S5

Ideal male mouse group size: The best things come in threes

Jirkof Paulin[1], Gaskill B.[2], Bratcher N.[3], Medina L.[3] and Ebert P.[3]

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Abstract

Social housing is recommended for mice; however, inter-male aggression can lead to injury and death. Here, we analyzed whether 1) smaller groups, and 2) earlier age of allocation to group decreases aggression and if 3) manipulation increases aggression in male mice. A 14wk study was performed to assess the following treatments in male CD-1/ICR mice: group size (1, 2, or 3), age at grouping (5 or 7 weeks of age), and manipulation (daily scruffing and weekly minimal handling). Wounding, body weights, food consumption, nest scores and sucrose consumption were documented. Finally, the pelt aggression lesion scale (PALS) was assessed.

Very little fighting was observed during daily checks. However group size significantly affected PALS. Pair-housed mice had

higher PALS and more overall wounding than groups of 3. Minimally handled mice also had higher PALS than daily scruffed mice. Surprisingly, solitary mice consumed more sucrose, suggesting they were less anhedonic. However, high nest scores and food consumption, and low body weights indicate solitary mice may be experiencing thermal stress, even with the provision of nesting material. While age of allocation altered our measures, there was no strong effect on mouse welfare. Based on this data CD-1 mice can successfully be housed for up to 14 weeks and groups of 3 may be the best for reducing even minor levels of aggression.

OC4S1

Ending severe suffering – Where are we now?

Reed Barney, Hawkins P. and Lilley E.
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Abstract
Each year across the European Union, almost one million scientific procedures are carried out on animals with an actual severity of 'severe'. This figure broadly represents an estimated 5-10% of all animal research undertaken. Procedures that are categorised as 'severe' are of significant concern to everyone involved in the regulation, care and use of animals in research – and to the public. There are both ethical and scientific imperatives to reach a point where no animal in research experiences 'severe' suffering.

Since 2012, the RSPCA has led a major initiative focussed on ending severe suffering. Working collaboratively with the scientific community in the UK and across Europe, we have initiated and promoted a range of pioneering activities aimed at identifying and promoting practical steps which will help people to reduce or, ideally, avoid 'severe' suffering.

At this FELASA Congress 2019, the RSPCA is co-organising three scientific sessions on the important theme of 'severe suffering'. This first, short presentation will consider the current scale of the challenge and progress made so far, and will set the scene for the talks and discussion which will follow throughout the day.

OC4S2

Acceptability of severe experiments in the mind-set of trainee laboratory animal users

Franco Nuno Henrique
Laboratory Animal Science, i3S – Instituto de Investigação e Inovação em Saúde, Porto, Portugal

Abstract
Public acceptability of animal experimentation is conditional on the purpose of research, species used, and respect for animal welfare. However, the extent to which such factors influence researchers' views on animal use remains unclear. As part of a broader study surveying attitudes of participants in laboratory animal science courses in four European countries, we asked respondenrs to classify the acceptability of hypothetical animal research projects of varying severity ('Mild', 'Moderate', 'Severe') and using different species (Rat, Pig, Dog, Rhesus macaque). As a 'severe suffering' case-study, dog experiments for 'testing of novel chemotherapeutic approaches for treating child leukaemia' were presented. Only 33.9% of respondents (76/224) deemed this project acceptable. Approval of severe studies in dogs was even lower for other scientific purposes, namely for 'testing novel drugs for treating obesity' (8.5% 19/224) and 'studying the influence of alcoholism in decision making' (4.5% 10/224). For leukaemia research on dogs, there was, however, a 72.8% approval below the 'severe' level, as 19.2% (44/224) would accept the project if suffering did not exceed a 'Moderate' severity classification, and 19.2% (43/224) if it would not exceed the 'Mild' classification. For leukaemia research, acceptability of severe studies using rats was 46.4% (104/224), which was higher than for all others species. The finding that most respondents did not find severe studies justifiable, even for experiments on rodents – typically of lesser ethical concern – and a scientific purpose socially seen in a favourable light, could mean a high receptivity to a move towards ending severe suffering in animal research.

OC4S3

Ending severe suffering – A view from a regulator

Ryder Kathryn
Home Office, Dundee, United Kingdom

Abstract
The regulator has a key role in minimising severe suffering during exchanges in the application process and during oversight of the work in the inspection programme, to ensure that continuous refinement is applied as new knowledge becomes available.

Each project is considered to ensure that all known opportunities for refinement to reduce severity are included before the procedures begin. In some cases, this will reduce the severity from severe to moderate, and could replace the model altogether. Regulators should ensure that the Animal Welfare Body effectively contributes advice on refinements in the planning phase, and reviews studies ensuring lessons learned are disseminated.

Regulators evaluate whether care staff and vets are providing appropriate advice during studies to reduce severity, and review circumstances where there has been unexpectedly increased severity to provide advice to minimise it.

A tiered and prioritised approach applied by regulators considers the severity of the authorised procedures and inspection frequency is increased when severe suffering is expected. This might also include procedures where there is an element of uncertainty.

All projects which have been authorised to include severe procedures must be subject to retrospective assessment. Information is necessarily provided on the harms which were suffered by the animals and any progress which has been made in applying the 3Rs. This gives the regulator the opportunity to reduce severity in future similar procedures and to disseminate knowledge to others.

OC4S4

Dutch prospective severity classification in 'severe' procedures

Verhave Peternella Suzanne
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Abstract
Severity classification is important in the discussion on animal welfare and refinement of scientific procedures. It is essential for the ethical consideration of procedures and it facilitates communication to society. Finally, prospective classification 'severe', strengthens protection of animals by mandatory retrospective evaluation, thereby the focus on humane endpoints, monitoring and inspection of these animals.

Differences in retrospective classification in statistics across EU member states, raises questions on severity classification. The Dutch relatively low percentages of 'severe' retrospective discomfort may be caused by the procedures used, optimal refinement thereof or an under estimation of discomfort. To structurally assess prospective classification, Dutch NTS (non-technical summaries) were analyzed (Dec 2014- Aug 2018). They contain information on prospective severity, procedures and refinements. The focus was on 13 examples of 'severe procedures' in directive 2010/63/EU.

Of 1231 NTS, 20% included the classification 'severe'. 98 NTS fitted within the 13 'severe' examples. Of those, 49 did not include 'severe' classification. Wording on negative consequences and humane endpoints didn't rule out the possible occurrence of 'severe' discomfort (in 25 out of 49). On the other NTS no conclusions on discomfort could be drawn.

The prospective classification alerts all involved to protect animals from unnecessary suffering. Today's strong emphasis on prospective assessment of scientific procedures does not guarantee correct classification according to directive 2010/63/EU. However, it's clear that the classification 'severe' is not structurally avoided in the Netherlands (20% of NTS). Let's cherish this ongoing discussion on severity classification to continue increasing animal welfare on site.

OC4S5

The scientific need to avoid uncontrolled and unalleviated suffering

Carbone Larry
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Abstract
Scientists have an ethical obligation to minimize laboratory animal suffering. Despite this, regulations and policies do allow scientists to cause pain and suffering in their animals, and to withhold some or all of the refinements that might reduce their suffering. Ethics committee reviews of projects with planned unalleviated suffering must look at several technical factors, and must make decisions even when the needed information is incomplete: what is the severity of the expected suffering? How will analgesics and other refinements affect the data from the experiments? Crucial, but less frequently addressed: how will unalleviated suffering affect the data? The ethical imperative to minimize animal suffering complements a scientific need to reduce suffering that could skew an animal's biological responses in experiments. For only a minority of models are gold standard studies available that compare data produced when animals are in significant pain and distress compared with those where refinements are rigorously applied.

This presentation reviews some ways in which unalleviated pain and distress can influence animal data, how scientists and ethics committees can find the best available information on the effects of analgesics and other refinements, and how the ethics committee should proceed when the necessary information on the model is not available.

OC5S1

Welfare assessment – Recognising and preventing severe suffering

Hawkins Penny, Lilley E. and Reed B.
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Abstract
The RSPCA has initiated and led a major initiative focussed on ending severe suffering since 2012, in collaboration with the scientific community throughout Europe. At this FELASA Congress 2019, the RSPCA is co-organising three scientific sessions on reducing, and ideally avoiding, severe suffering. This second session will focus on 'Welfare assessment – recognising and preventing severe suffering'. This introductory presentation on 'Available resources to help assess and prevent severe suffering' will briefly outline available resources and guidelines that can help to facilitate effective welfare assessment, so that severe suffering can better be prevented and reduced. For a copy of the presentation, please email research.animals@rspca.org.uk

OC5S2

Welfare assessment as a tool to avoid severe suffering in mice and fish

Riederer Beat
Editior-in-Chief LA; c/o Plateform of Morphology and Department of Psychiatry, Hospital of the Canton Waadt (CHUV) and University of Lausanne, Lausanne, Switzerland

Abstract
Avoiding severe suffering is one of the most difficult calls in animal experimentation with focus on the detection of pain and reducing suffering. Currently, severity degrees are classified from 0 – 3, with degree three having most constraints regarding suffering, pain and distress. For licensing of experiments such a classification is essential, including a harm-benefit analysis. In addition, a retrospective evaluation is also important for reporting of the actual constraint. However, such an evaluation asks for an

accurate discrimination between pain levels. But, is it possible to distinguish different degree of pain in animals? Expression of pain varies between species and experimental models. Therefore, it is essential to know what to look for. Much is based on observation. It is also essential to establish a list of criteria or score sheets to interrupt experiments when animals are moribund, and to define humane endpoints in order not to lose valuable data, by premature deaths. Such criteria may be used to verify if a given analgesia is effective or more analgesia is needed. Therefore, it is important to reinforce education for all personnel that is handling animals, how to distinguish between different types of suffering. For rodents, observation criteria are well reported and can be applied – when you know what to look for. As many vertebrates, fish have also pain receptors and produce endorphins; they change their behavior after insult or when exposed to noxious stimuli, but to discriminate between different pain levels is more difficult, and needing more refinement.

OC5S3

Multifactorial severity assessment: Grading animal distress in a murine pancreatic cancer model

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Abstract
Introduction: Severity assessment of animal models is required for pursuing animal research in the European Union. However, studies with detailed distress assessment in animal models, or recommendations how to pursue multifactorial distress analysis are still rare.
Material and Methods: We therefore established a multifactorial distress analysis, by assessing physical, behavioral and hormonal distress parameters at different time points, after induction of pancreatic cancer in an orthotopic mouse model and during two different chemotherapeutic treatments. A grading of distress was achieved by 3D cluster analysis and Support Vector Machine classification.
Results: The single distress parameters burrowing behavior and faecal corticosterone metabolite concentration, proved to be significantly more accurate in predicting distress after surgery than body weight change. However, the multifactorial approach, using all three parameter revealed the highest accuracy for distress prediction. This three dimensional distress analysis, indicated increased distress after surgery followed by a fast recovery of the mice within one day. Moreover, we quantified that the chemotherapy of metformin plus galloflavin caused significantly more distress compared to the metformin plus α-cyano-4-hydroxycinnamate treatment. In addition, a desensitization to the applied drugs was observed.
Conclusion: This multifactorial approach for distress assessment proved to be a good tool to grade animal distress after different interventions and to judge the side effects of drugs.

OC5S4

Evaluation of animal distress in a cerulein-induced chronic pancreatitis animal model

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[2]*Unit of Physiology, Pathophysiology and Experimental Endocrinology, Department of Biomedical Sciences, University of Veterinary Medicine Vienna, Vienna, Austria*

Abstract
Introduction: Chronic pancreatitis induced by repetitive injection of cerulein represents a very reliable and widely used animal model. In accordance with EU-legislation, the scientific community is, therefore, in crucial need to evaluate the distress associated with this animal model.
Material and Methods: We compared distress caused by chronic pancreatitis to distress caused by laparotomy relying on the specification of European Directive 2010/63/EU that laparotomy causes moderate distress. We assessed body weight change, burrowing, nesting activity and faecal corticosterone metabolites concentration before as well as after laparotomy or induction of chronic pancreatitis in C57BL/6J mice. We used receiver operating characteristic curve (ROC) analysis, to select the optimal combination of parameters and calculated Youden's index to define the optimal cut off to differentiate between distress and no distress using data before and after laparotomy. The optimal cut off was then applied to the pancreatitis data set and a second laparotomy data set with subsequent comparison of distress in both animal models.
Results: Significant changes in all distress parameters were observed after laparotomy and after induction of chronic pancreatitis with no mortality. ROC analysis revealed a higher accuracy of multi-parametric analysis in distress assessment. Interestingly, when comparing chronic pancreatitis to laparotomy, significantly fewer data points indicated distress during chronic pancreatitis. Thus, at any time point, mice with chronic pancreatitis experienced less or similar distress as mice after laparotomy.
Conclusion: We suggest that distress experienced by mice at any time point during cerulein-induced chronic pancreatitis can be defined as mild to moderate.

OC5S5

Severity Assessment after partial liver resection in rats

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Abstract
Introduction: Severity assessment is based mostly on subjective parameters. Objective parameters to assess severity for experimental animals are urgently needed. Study aim was to evaluate the severity of a 50% liver resection in rats. The influence of different sealants (fibrin glue, polyurethane glue (PUG) & saline control) on the liver was compared. The severity was evaluated with the open field (OF) & clinical scoresheet (Morton DB et al 1985).
Methods: 63 male WISTAR rats (250–300 g) were randomly assigned to 3 groups (n = 21/group; 1day, 3days, 7days). In 7day-survival group 12 animals were implanted with telemetry transponders under general anesthesia & analgesia. Partial liver resection of 50% was performed after recovery time. At all surgical procedures rats got pre-op Metamizole 100 mg/kg BW s.c. & 400 mg/kg via sweetened drinking water for 3 post-op days (POD). OF was carried out on day 1, 3, 4 & 7.
Results: OF showed that all animals moved the shortest distance at POD1 and reached their baseline values at POD7 at latest. Bodyweight analysis showed lowest BW at POD2 and recovery to baseline latest at POD4. Pre-analyses of blood pressure showed significance between groups. Further analyses of heart rate & activity during behavioral tests compared to resting phases are in progress. Clinical score (0–20) showed highest values on POD2 (PUG 4.21 ± 3.02, Fibrin 3.02 ± 2.82, NaCl 2.86 ± 2.26 ns).
Conclusion: The results of this study show that the severity after 50% liver resection is mild to moderate. However due to the EU Directive it is classified as moderate.

OC6S1

The marginal gains concept: Shifting from disease models to mechanistic models

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Research Animals, Royal Society for the Prevention of Cruelty to Animals, Southwater, United Kingdom

Abstract
Since 2012, the RSPCA has led a major initiative focussed on ending severe suffering. Working collaboratively with the scientific community in the UK and across Europe, we have initiated and promoted a range of pioneering activities aimed at identifying and promoting practical steps which will help people to reduce or, ideally, avoid 'severe' suffering. At this FELASA Congress 2019, the RSPCA is co-organising three scientific sessions on the important theme of 'severe suffering'. In this third session we will focus on 'Practical refinements of animal models and procedures that may cause 'severe' suffering', to showcase examples of ways in which these can be refined to avoid or reduce suffering. In this short introductory presentation I will outline two principles that can be used to achieve this – firstly, the 'accumulation of marginal gains' concept, and secondly, a shift from animal models of disease to animal models of mechanism. The latter can both reduce suffering and pave the way towards greater use of innovative replacement technology.
www.rspca.org.uk/severesuffering

OC6S2

How severe procedures have been refined in diabetes research

Bertelsen Thomas
Animal Bioethics, Novo Nordisk A/S, Maaloev, Denmark

Abstract
Addressing severe suffering is relevant and required for legal, scientific as well as animal welfare reasons, – diabetic and metabolic research being no exception.

The presentation will describe in which scientific circumstances severe suffering occurs in diabetes research as well as the challenges associated with eliminating or reducing the severity in this research field.

The presentation will focus on procedures that have been introduced and are in place at Novo Nordisk. These include husbandry measures as well as scientific interventions. Having diabetes or a metabolic condition is not just one single and well-defined clinical disorder, and consequently the animal models must cover a wide range of clinical expressions and scientific endpoints to be able to reflect all of these. To address this requirement for diversity and the associated challenges in terms of potential severe suffering, the talk also presents the Global Pharmacology Council, which we have recently introduced at Novo Nordisk, and give examples on how this supports good science as well as ensuring animal welfare.

Finally, the presentation gives an insight into a new technological tool that will help eliminating or reducing severe suffering in diabetes research.

OC6S3

Avoiding mortality in animal research and testing

Lelliott Chris
Wellcome Sanger Institute, on behalf of a working group convened by RSPCA, the Laboratory Animal Science Association (LASA), Laboratory Animals Veterinary Association (LAVA) and the Institute of Animal Technology (IAT), Cambridge, United Kingdom

Abstract
There are legal, ethical, animal welfare, and scientific reasons to avoid mortality in animal research and testing. For example, Directive 2010/63/EU requires death as an endpoint to be avoided as far as possible and replaced by humane endpoints. Actual severity is also assumed to be severe if an animal is 'found dead' (unless an informed decision can be made otherwise). As part of an ongoing joint initiative to reduce and avoid severe

suffering for laboratory animals, the RSPCA, LASA, LAVA and the IAT set up a working group to identify ways of avoiding:

- unpredicted mortality in stock animals held for future breeding or experimental use, both wild type and genetically altered;
- unpredicted mortality in animals undergoing procedures; and
- predictable mortality of animals, for example in studies to fulfil those regulatory requirements where, currently, death is explicitly required.

This presentation will provide an overview of the group's recommendations for good practice approaches to predicting and avoiding death, including reviewing welfare assessment; undertaking pilot studies; improving staff training; data or record mining; and reviewing and challenging regulatory requirements. The group also addressed issues needing careful consideration, e.g. interpreting indicators of impending mortality in aged animals, and considering harms and benefits of increasing surveillance when this might cause additional distress. The resulting guidance document is freely available and designed to be applicable throughout the EU.

OC6S4

Severity assessment and pain management in the acute Cerulein-induced pancreatitis mouse model

Durst Mattea[1], Arras M.[1] and Jirkof P.[1,2]
[1]*Division of Surgical Research, University Hospital Zurich, Zurich, Switzerland*
[2]*Department Animal Welfare, University of Zurich, Zurich, Switzerland*

Abstract
Mouse models of acute pancreatitis are of importance in translational research. While pancreatitis is painful in humans, little is known about the level of pain in the acute mouse Cerulein model. As a result, severity classification is discussed controversially and pain monitoring is not standardized. Frequently analgesia is withhold in this model, which could result in decreased animal welfare. Here, we aim for a scientific based severity assessment in the acute pancreatitis model and attempt to provide specific analgesia regimes lacking detrimental side effects on experimental read-out.

We investigated an acute pancreatitis in male C57Bl/6 mice to detect pain and decreased wellbeing. Cerulein, a peptide commonly used to induce pancreatitis in rodents was injected intraperitoneal 12 times during two consecutive days. Mice injected with NaCl in the same time regime served as a control. We measured body weight, food and water intake. We applied the burrowing test, mouse grimace scale and the von Frey test to detect pain and hypersensitivity in the abdomen. Fecal corticosterone metabolites were measured to outline short-term stress. Additionally, pancreases were histologically examined to grade inflammation. In a second setup, we applied different analgesics (Paracetamol: Tramadol mixture, Metamizole, Buprenorphine) via the drinking water and tested them for efficacy, side effects and influence on the experimental model with the mentioned parameters.

Experiments are currently still in progress and full results will be presented at the conference. Preliminary results hint on a low level of pain, several indicators to detect pancreatitis pain in mice were identified.

OC6S5

Characterization of a novel technique to perform laminectomy in spinal cord injury rat model

Vijayakumar Sreelatha Harikrishnan[1], KK L.[2] and Abelson K.[3]
[1]*Department of Applied Biology, Division of Laboratory Animal Science, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Bio Medical Technology Wing, Trivandrum, Kerala, India*
[2]*Department of Applied Biology, Division of Thrombosis Research, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Bio Medical Technology Wing, Trivandrum, Kerala, India*
[3]*Department of Experimental Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*

Abstract
This study aimed at developing a novel motorised technique to perform thoracic (T10) laminectomy to induce spinal cord injury (SCI), and investigated the effects of this technique on pain and overall animal welfare and on the outcome of the animal model. A total of 24 female rats (Crl:WI; n = 6 per group), 240–280 g, 9–12 weeks of age were used and the animals were divided into four groups: Conventional laminectomy without SCI (Group I),laminectomy without SCI using motorised dental burr assisted (DBA) technique (Group II), SCI using a conventional approach (Group III) and in Group IV (n = 6) SCI using a DBA approach. The animals were housed in individually ventilated cages with 12:12 light:dark cycles not exceeding 325 Lux at 1 m height from floor level and fed with commercial rat feed and purified water *ad libitum*. Pre-and post-surgical behavioural scores like rearing, activity, Basso Beattie Bresnahan (BBB) score, Rat Grimace Scale (RGS) were recorded and compared between groups. The effect of novel technique in above-mentioned aspects were compared to conventional laminectomy using a battery of clinical and behavioural tests. Body weights, anhedonia using 1.5% sucrose preference, novel object recognition in acute and chronic phases, open cage activity, Von Frey mechanical nociception, motor functionality assessment using BBB score and home cage activity during the dark phase were monitored until the 9th week postoperatively. The results indicate that, the novel technique has a positive impact on the welfare and well being of SCI induced rats, without affecting the outcome of the animal model.

OC7S1

Analgesia in laboratory rodents – Current paradigm and future perspectives

Abelson Klas
Department of Experimental Medicine, University of Copenhagen, Copenhagen, Denmark

Abstract
Pain in rodents in relation to invasive procedures is problematic from an animal welfare as well as from an experimental perspective. Thus, accurate recognition, assessment and alleviation of pain in these animals is essential. However, pain relief in rodents is not problematic. Difficulties to recognize occurring pain properly may impair the accuracy of the treatment, or lead to lack of treatment as well as overdosing, depending on the situation. Furthermore, the potential adverse effect of pain medication on the experimental outcome may complicate the treatment strategies even further.

This lecture will discuss these challenges in detail and present an overview of the current paradigm as well as novel findings on how to implement analgesic strategies for optimal pain treatment. The presentation aims to provide guidance as well as inspiration for scientists, veterinarians, animal caretakers and technicians and others involved in invasive procedures on rodents.

OC7S2

Same old story? Same colour, same look but otherwise nothing is the same

Petrie Anja
Medical Research Facility, University of Aberdeen, Aberdeen, United Kingdom

Abstract
This presentation will cover the experience as Named Veterinary Surgeon of dealing with three strains of genetically altered mice used as models of dementia and Alzheimer's research which appear to have an increased pain sensitivity when undergoing surgery for brain electrode implantation.

All three of the genetic altered mouse strains are on the same background strain (NMRI). The animals were surgically prepared with brain electrodes to allow measurement of brain activity post recovery from surgery in free moving animals using several different behavioural tests. Increased pain sensitivity has been reported in humans suffering from Dementia or Alzheimer's. Our experience would indicate that this increased sensitivity is present in these mouse models.

The presentation will discuss how animal welfare post- surgery was improved by a range of measures: combining different types of analgesics, extended provision of pain relief (including offering pain relief prior to surgery), and by the consideration of different dosages and routes of administration. It will discuss how improved peri-operative care measures helped a better recovery. The presentation will also cover the challenge of increased anxiety in at least one of the strains and success/limitations addressing this.

OC7S3

Multimodal pain management with local anesthesia – A chance for refining surgical mouse models?

Durst Mattea[1], Arras M.[1] and Jirkof P.[1,2]
[1]*Division of Surgical Research, University Hospital Zurich, Zurich, Switzerland*
[2]*Department Animal Welfare, University of Zurich, Zurich, Switzerland*

Abstract
Local anesthesia (LA) as part of multimodal pain management has the potential to decrease post-surgical pain perception and hypersensitivity around the wound site. Recommendations on the use of LA in mouse surgery exist but systematic studies on the efficacy and side effects of LA in mice are lacking. A possible reason why LA is not often used in surgical mouse models. As a result, a refinement measure could be missed.

In a laparotomy model in C57Bl/6J mice, we assessed possible benefits of Lidocaine-Bupivacaine infiltration in combination with systemic Paracetamol applied via drinking water compared to systemic analgesia only. Following groups were compared: Surgery with LA and Paracetamol, and surgery with LA or Paracetamol only. One group without surgery received anesthesia and Paracetamol only. Lidocaine–Bupivacaine was injected subcutaneous around the surgical site two minutes prior to the incision. We measured body weight, food and water intake and nest complexity to depict changes in animals' wellbeing and potential pain. We analyzed sugar consumption to detect anhedonia. Additionally, we assessed perceived pain with the burrowing test, the Mouse Grimace Scale and tested for mechanical hypersensitivity around the wound area with the von Frey test. We analyzed fecal corticosterone metabolites to depict short-term stress.

Analyses are still ongoing, full results are presented at the conference. Preliminary result show that mice ingested sufficient amounts of Paracetamol. No obvious differences between groups, no wound healing disorders and other side effects were observed with LA treatment.

OC7S4

Refining aseptic conditions in rodents surgeries: The way forward

Bouard Delphine
Surgery, Vetsalius, Saint Didier au Mont d'Or, France

Abstract
Strict aseptic surgery principles are generaly applied in private and public institutions performing surgeries in large laboratory animal species (non human primates, dogs, pigs and minipgs, goats for example). However, despite the fact that aseptic conditions are mandated (EU directive), they are still not extensively applied in small animals and especially in rodents.

Different explanations could be advanced. The same person often serves as surgeon, anesthetist, surgical technician when

surgical procedures are performed on rodents. Current rodent facilities organization and especially surgery room laying out are often suboptimal in terms of aseptic conditions. For some surgeries, devices such as binoculars or sterotaxic frames should be used and these items are very often difficult to sterilize or manipulate without compromizing aseptic conditions. Experimental design frequently requires repetitive surgery, which enhance the risk of contamination. Last but no least, many "rodent surgeons" didn't have the opportunity to follow extensive aseptic conditions training, which is the case of most large animal surgeons.

These difficulties could and should however be overcome, as accidental contamination during surgery could significantly affect research projects relevance and reproducibility. When it comes to experimental surgery, survival alone could definitely not be considered as a validation criterion (Cunliffe-Beamer, 1993).

The second part of the presentation will give an estimation of the current status of aseptic conditions in rodents "level" in EU and will give information regarding how to help, through proper training and practical "tips and tricks", research teams to keep on refining aseptic techniques in small laboratory animals.

OC8S1

Kleiber's Law: Help or hindrance

Tobin Graham
Retired, Banbury, United Kingdom

Abstract
It seems intuitive that bodyweight should contribute significantly to energy and nutrient requirements and organ size in animals. The problem is how one might best define such a relationship. In the 1930's Kleiber advanced the idea that metabolic rate (and the requirement for many nutrients) was empirically related within and between animal species by bodyweight raised to the ¾ power ($W^{3/4}$, now usually expressed as $W^{0.75}$). Adjusting for body size by applying $W^{0.75}$ has become common in energy and nutrient metabolism and in many physiological measurements. However, investigators rarely consider whether 'normalising' or 'standardising' data to $W^{0.75}$ is valid.

There are several problems with the adoption of $W^{0.75}$ to standardise for differences in body weight in laboratory animal studies: (a) Kleiber's relationship was defined for mature animals only, yet most experimental situations in which it is used involve young growing animals; (b) the relationship is empirical with no immediately obvious physiological basis; and (c) in recent years, further regression analyses have largely discredited a universal exponent of 0.75, including for laboratory animals.

Adjusting data to $W^{0.75}$ may not only be incorrect but may obscure real nutritional and physiological relationships. This may be particularly true when it is used to 'standardise' values between lean and obese animals. Examples of both weaknesses are given. Unless investigators are able to explain and justify such adjustment of data, 'standardisation' should be avoided. Statistical methods such as ANCOVA might be a better way of compensating for body-size variation.

OC8S2

Defining accurate protein and amino acid requirements in laboratory rodents

Afonso Ricardo A.[1], Schuhmacher A.[2] and Tobin G.[3]
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[2]ssniff Spezialdiäten GmbH, Soest, Germany
[3]Convener of the FELASA Working Group on Nutrition of Laboratory Rodents, Bodicote, United Kingdom

Abstract
Accurate knowledge of protein and amino acid requirements in laboratory animals is important. While the deleterious effects of inadequate levels of proteins and amino acids are well known, excessive levels may also have adverse consequences. Although still debated, excessive dietary protein may contribute to chronic nephropathy and decreased lifespan, amongst other conditions. Inappropriate levels of individual amino acids may also be deleterious.

The NRC published estimates of protein and amino acid requirements of laboratory rodents over 20 years ago, but with few systematic studies on which to base its proposals. Consequently, its recommendations are incomplete, rarely based on systematic dose-response relationships, and applicable to purified diets only.

Our working group aimed to achieve, as part of its remit, accurate definitions of protein and amino acid requirements for growth and maintenance in a systematic way for purified and natural-ingredient diets. Three major factors impinge on our estimates: dietary energy content; dietary amino acid composition relative to requirement; and amino acid bioavailability. Few studies we examined considered all these factors, but we were able to compensate appropriately for them. We adopted a different approach to maintenance requirements. NRC's estimate represents the protein and amino acids required when there is no net protein deposition. While of academic interest, this does not represent the practical situation in rats and mice which, unusually among animals, continue to grow for the whole of their life. Our data also provides appropriate maintenance estimates for the first time.

OC8S3

Experimental diets – Challenges in rodent experiments

Schuhmacher Annette
ssniff Spezialdiäten GmbH, Soest, Germany

Abstract
Laboratory animal diets are used for husbandry and experimental purposes. As experimental diets, they are used to manipulate nutrient levels (e.g. high fat diets) or as a carrier for substances. Most are manufactured from purified ingredients that give accurate and precise nutrient control. However, natural-ingredient experimental diets are sometimes used to reduce cost. They also may act as a carrier for compounds and nutrients such as fat. While they are acceptable for small additions of materials, high

levels of nutrients or other materials may produce an imbalance in nutrient composition and/or unexpected side effects. In contrast, purified diets are easy to adjust to compensate for large additions, preventing imbalances or deficiencies.

Natural-ingredient diets are commonly used as the control for purified-diet experimental groups. However, they differ from purified diets in many aspects that might confound the experimental outcome. They may contain physiologically active phytochemicals not present in the experimental groups and recently the potential for their high levels of soluble and insoluble fibres to affect the microbiome has been recognised.

Although analytically a natural-ingredient diet may appear like the desired purified control diet, its nutrient digestibility and utilisation are considerably lower. For example, only about 60 to 80% of dietary protein, amino acids, and some minerals (particularly phosphorus) are available to monogastric laboratory animals from natural-ingredient diets.

Diet cost should not be allowed to compromise reproducibility. Purified experimental diets are flexible, and accurate and precise in formulation. Absence of confounding bioactive substances is a major advantage over natural-ingredient diets.

OC8S4

Diet can affect data interpretation and reproducibility

Pellizzon Michael and Ricci M.
Research Diets, Inc., New Brunswick, NJ, United States

Abstract
Preclinical research is essential for understanding our biological processes, but is becoming more difficult to interpret and reproduce. There are many reasons for this, but one not considered very often is the diet fed. Two diet types are commonly used: Grain-based (GB) diets and purified diets. GB diets are closed (proprietary) formulas containing various grains and animal by-products, each of which provides multiple nutrients/non-nutrients. In contrast, purified diets are open formulas and each refined ingredient provides one main nutrient. Non-nutrient contaminants found in GB diets include phytoestrogens, heavy metals, mycotoxins, endotoxins, pesticide residues, and pollutants; in contrast, these contaminants are virtually absent in purified diets (e.g. Dioxynivalenol varied from 524 to 1,012 ppb in GB diets; not detectable [detection limit 100 ppb] in purified diets). In addition, due to the use of unrefined grains and milling by-products, the types and levels of nutrients in GB diets – such as fiber – differ greatly from purified diets. GB diets contain relatively high amounts of multiple fiber types (e.g. hemicelluloses, lignins, pectin) whereas purified diets commonly contain one fiber source (e.g. cellulose). Too often, GB diets are used as controls for purified diets and such comparisons make data interpretation impossible, given their very different compositions. These comparisons and the presence of variable and numerous contaminants in GB diets likely play into the current reproducibility crisis in biomedical research and require more attention. This presentation will provide informative examples of how diet choice can affect data interpretation and data reproducibility in rodent models.

OC8S5

Dietary phytoestrogen disrupts the breeding output of mice although the embryo quality remains unaffected

Helppi Jussi[1], Naumann R.[1] and Zierau O.[2]
[1]*Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), Dresden, Germany*
[2]*Institute of Zoology, Technische Universität Dresden, Dresden, Germany*

Abstract
With an increasing need to constantly produce more transgenic strains it has become essential to focus even more on production and breeding efficiency with the aim of producing more strains with fewer mice. One potential way to increase breeding efficiency is to use optimal diet. One of the commonly used protein source in rodent diets is soy, which is naturally rich in phytoestrogens. Although phytoestrogens have shown potential health benefits in humans, they may also have the ability to disrupt mouse reproduction. Consequently, there have been a tendency to try to exclude them from rodent diets. In the current study we investigated whether high or low phytoestrogen content in mouse diet could affect mice used as embryo donors. Donor mice were maintained with three different diets – high phytoestrogen, low phytoestrogen and standard breeding diet. The high phytoestrogen diet resulted high yield of embryos, good quality of embryos, and high yield of injectable embryos. Throughout the experiments the results from low phytoestrogen fed donor mice were only slightly inferior, whereas mice fed by standard diet performed the poorest. Most interestingly, the majority of born and weaned offspring were observed when recipient females received embryos from standard diet group. We conclude that for experimental endpoints requiring fertilized embryos it may be more beneficial to feed mice with a diet containing phytoestrogen, but if the goal is to produce transgenic mice high phytoestrogen content in diet seem to be inadvisable.

OC9S1

Visualizing in vivo host-pathogen interactions

Le Grand Roger
IDMIT, CEA, Fontenay-aux-Roses, France

Abstract
Host-pathogens interactions occur in complex microenvironments characterized by rapid dynamic changes, the compartmentalization of highly specialized immune and stromal cell populations, and the presence of a complex network of soluble factors. Imaging platforms have provided critical contextual information regarding the molecular and cellular interactions that orchestrate the spatial microanatomy of relevant cells and the development of immune responses against pathogens. New technologies are becoming available to explore these interactions directly in large living animals and humans, from microscopic level to whole body visualization. Studies NHP models, can strongly benefits from in vivo imaging approaches: 1) for tacking vaccine antigens and

therapeutics in infected hosts; 2) for visualizing host responses to vaccination and treatment, with a specific focus on dynamics of immune effectors' changes: 3) for tracking microbes transmission and dissemination in the host; 4) for refining and reducing the use of animal models for infectious diseases preclinical research. Several of these technologies have also strong potential for translation to the clinical research and practice in humans. Finally, merging imaging platforms with other cutting-edge technologies could lead to novel findings regarding the phenotype, function, and molecular signatures of particular immune cell targets, further promoting the development of new treatments and vaccination strategies. The talk will focus on several examples of in vivo imaging approaches for studying vaccine response and mechanisms of infection in NHP models of human infectious diseases.

OC9S2

PET-CT tuberculosis imaging in non-human primates

Stammes Marieke, Haanstra K., Vervenne R., Vierboom M. and Verreck F.
BPRC, Rijswijk, Netherlands

Abstract

Introduction: In a constant effort to refine the use of NHPs in biomedical research and additionally gain more information per individual animal, it is a pertinent goal to pursue the recording of disease dynamics by minimally invasive methods beyond classical pathology assessment. PET-CT provides such a method as it enables in a minimal invasive way the visualization of anatomical and functional features. PET-CT in combination with ^{18}F-FDG is widely used in the clinic and has opened also opportunities in NHP research. The aim was to show the accuracy of ^{18}F-FDG PET-CT versus lung pathology in NHPs in a tuberculosis model.

Methods: PET-CT was performed at two-weekly intervals from one month after experimental tuberculosis infection onward. Images were obtained using a preclinical MultiScan LFER 150 PET-CT (Mediso Medical Imaging Systems) on anesthetized rhesus macaques (n = 24). All CTs were acquired with a breath-hold with and without CT contrast (Omnipaque 300, 2 ml/kg).

Results: In total 80 scans were recorded from which we determined tuberculoid lesion size by CT and the uptake of ^{18}F-FDG in those lesionsby PET. In retrospect, we performed a correlation analysis withthe lung pathology score at study endpoint. Correlation coefficients ranging from 0.72 to 0.91 over time were highly significant.

Conclusions: Our results demonstrate the utility of PET-CT to monitor tuberculosis infection dynamics in a minimal-invasive manner over time. Combining imaging with pathology assessment, we shall gain a deeper understanding of TB pathogenesis and protective or curative experimental treatment in this clinically relevant NHP model.

OC9S3

Stereotactic Surgeries and MRI-guided Convection-Enhanced Delivery (CED) into the striatum in Cynomolgus Macaques

Luft Jörg[1], von Keutz A.[1], Runge F.[1], Voß T.[1] and Grote-Wessels S.[2]
[1]*Animal Welfare and Comparative Medicine, Covance Preclinical Services, Münster, Germany*
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Abstract

Gene therapy is a promising area of drug development for a number of diseases including neurological disorders. Cynomolgus macaques (M.fascicularis) show high nucleotide sequence homology with humans. An IACUC-approved regulatory toxicity study required bilateral administration of gadolinium-labeled viral vector into the Striatum (Caudate nucleus and putamen) with temporarily implanted catheters. Magnetic resonance imaging (MRI) was used for calculation of trajectory and for surveillance of test article delivery during convection-enhanced delivery. 24 Macaques were prepared, induction of anesthesia with ketamine / medetomidine, intubation, clipping, disinfection, placement in a stereotactic frame, inhalation anesthesia (isoflurane). Animals were transferred to MRI to obtain data for catheter placement, assuring that the trajectory will not cross vessels/ventricles. The stereotactic frame was used for correct placement of catheters based on the MRI-coordinates. Skin was reclined from skull. Holes were carefully drilled. Catheters were cut to specific length and inserted along calculated trajectories to administer viral vector to 4 different locations into the Striatum. Catheters were connected to infusion lines. Infusion (rate 0.3 mL/h) was monitored in MRI for 80min to verify correct administration into the Striatum. Thereafter animals were transferred back to the surgical suite and catheters were explanted. Animals recovered from the 6-8h anesthesia within 15–30min and were treated with analgesics and antibiotics.No animal showed neurological abnormalities. Two animals presented with laryngeal swelling were treated with corticosteroids successfully.

Conclusion:

- 96 catheters successfully implanted in 24 animals
- Fast uneventful recovery from 6-8 h isoflurane anesthesia
- Successful intracranial administration into Striatum (Caudate nucleus / Putamen) utilizing CED

OC9S4

Novel model for activity and behaviour assessment in freely moving, group-housed Macaques

Bergmann Caroline[1], Dale R.[2], Collier K.[2], Devos M.[2,3] and Austin R.[2]
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[2]*Experimental Psychology, University of Oxford, Oxford, United Kingdom*
[3]*Biomedical Engineering, University of Oxford, Oxford, United Kingdom*

Abstract
Effective welfare assessment of NHPs used in biomedical research is an ethical paramount to optimise the implementation of the 3Rs. Here, we present pilot data using non –invasive accelerometers to assess the welfare impact of various life time events. This novel method is involving soft, microfiber neck collar individually designed and fitted for rhesus macaques, which contains a compact, lightweight piezo- electric accelerometer. The data collected can be used to analyse two (i) physical activity and diurnal rhythm (ii) home cage behaviour.

Physical activity is a well-known and important contributor to psychological and physical health; continuous measurement of an animal's activity levels and patterns can provide key insights into their welfare state. Activity budgets are variable between individuals; various factors such as age, body weight, social group structure and housing arrangements appear to contribute to individual animal's activity patterns and levels.

Behavioural model predictions were made using a decision tree algorithm, characterising data on the basis of mean bout amplitude, bout duration and bout amplitude variation. The model provides a semi-automated behaviour monitoring tool allowing to distinguish between 5 different classes of behaviour (periods of rest/ inactivity; social interaction/ foraging; pacing; walking; running/ jumping). Bespoke algorithms have also been developed for sleep analysis.

Together, both methods allow synchronised continuous and observer -independent data collection with a high spatial resolution. Accelerometry appears to be a powerful method to gain further insight into non NHP life-time experience and wellbeing with potential to further enhance the applications of the 3Rs in biomedical research.

OC10S1

Efficient ways to responsibly use genetically altered laboratory rodents

Jerchow Boris
Research Animal Facilities, University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Gibraltar

Abstract
Numbers of animals used in scientific procedures remain fairly constant over the years. However, experimental animals that are bred and culled without being used had to be reported to the EU commission for the first time in 2018. It is not surprising that this pertains predominantly to genetically altered rodents. Current concern focuses mainly on the reception of these numbers in the wider public without addressing the roots of the issue. While there is some awareness of the existing shortcomings, especially in the generation, breeding, and use of genetically modified mice, the established systems appear reluctant to change. While the calls for reorientation are becoming louder, we need to consider that it is nothing less than a change in mindset that needs to be achieved. Since legal restrictions will only work through laborious, unpleasant, and embarrassing control systems, we need to incentivize and show efficient ways to a responsible use of laboratory animals.

OC10S2

Impact of recent technological updates on the 3Rs in high throughput mouse production

Doe Brendan
Welcome Sanger Institute, Hinxton, United Kingdom

Abstract
We now know through sequencing of the mouse genome, that there are approximately 20,000 protein coding genes in the mouse. International collaborations such as the International mouse phenotyping consortia (IMPC) seek to discover functional insight for each of these genes by generating and systematically phenotyping knockout mouse strains. High throughput production and archiving in a pipeline are required as part of this effort. Here we examine the impact of new technologies and refining processes on the 3R's in this setting.

OC10S3

Positive impact of 3R focused GAA rodent breeding

Gomas Emmanuel
EGSconseil, Baillargues, France

Abstract
Between line creation and phenotypic evaluation of a GAA rodent line, producing experimental batches for phenotyping is a mandatory step which can influence dramatically the full course of the project impacting results reproducibility.

As highlighted by The Netherland authorities ''bred but not used'' studies in 2013, this batch production phase can lead to massive production of useless animals, not only because of improper genotypes produced during multitransgenic animals breeding. As well, improper genetic background management or lake of environmental parameter control is at the origin of results variability.

By having a 3R oriented GAA rodent breeding strategy, it is possible to improve cost and timeline related to batch production, while improving also result reproducibility and reduce dramatically the number of animals produced.

Cryopreserving all GAA lines may allow to stop breeding lines not used for more than 8 months. Sperm freezing and IVF is a solution to avoid maintaining reporter lines or recombinase lines without impacting timeline of multitransgenic models while saving lot of cages and animals. Proper backcrossing and genetic drift management leads to lower results variability and may avoid production of useless litter mates. A priori calculation of breeding level as well as proper genotyping process allows reduction and refinement in project management.

Starting from case studies, this session aims to remind proper use of these key colony management tools and will highlight savings made both on financial and welfare point of view.

OC10S4

Refinement as an aid to improving breeding outcome

Thomas Aurelie
Wellcome Sanger Institute, Hinxton, United Kingdom

Abstract
Production, expansion, and management of GAA colonies implies putting individual animals through a succession of technical procedures. Each of them has the potential to influence the welfare of the animal as well as the breeding outcome.

This presentation will focus on ways to refine mating strategies, embryo transfer and genotyping techniques. For each technique, the impact on the breeding outcome at the level of the colony will be discussed where published.

OC10S5

Times up for tick-over colonies...

Woodley Stephen and Newman S.
Biological Services Unit, King's College London, London, United Kingdom

Abstract
The practice of continually breeding lines in order to maintain them as a live resource either in-between studies or indefinitely ''just in case'' is common practice in many animal facilities. This process of ''ticking over'' colonies has historically occurred due to the unavailability or unreliability of archiving services, coupled to the economic cost associated with the processes. The advent of reliable sperm cryopreservation and associated recovery processes has now eliminated most reasons to not archive lines.

The creation of a genome editing and embryology core within King's College London Biological Services has permitted rapid and efficient archiving which in turn has led to the substantial reduction in ''tick-over'' colonies. Between September 2015 and October 2017 various subsidy incentives were offered to research groups resulting in over 350 lines being cryopreserved with 97 removed as a live resource. This is a significant ethical refinement as it is estimated to have prevented the breeding of 4000 mice per year. Additional benefits include additional space becoming available for scientific research and the introduction of new lines.

The culture throughout small laboratory animal establishments to maintain ''tick over'' colonies has been successfully challenged by subsidised and targeted archiving throughout KCL Biological Services and has in turn facilitated further scientific work. Incentivised archiving is a noteworthy indication of our commitment to implementing the 3R's across King's College London.

OC11S1

The evidence base for refined methods of handling mice

Hurst Jane
Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom

Abstract
Routine handling of laboratory animals is an essential but frequently ignored component of animal experiments that has considerable potential to influence anxiety and aversion to human approach and contact. Studies from our laboratory have shown that the method used to pick up laboratory mice is critical. Picking up mice by the tail induces aversion and high anxiety (even if their weight is supported), whereas use of handling tunnels or scooping mice up on the open hand (cupping) leads to voluntary approach to handling, low anxiety and acceptance of physical restraint. These responses appear to be quite consistent across strains and sexes of mice, across different laboratories, across handlers with different levels of prior experience, and whether animals are handled in the light or dark phases of the diurnal cycle. Mice picked up using a handling tunnel or scooped on the open hand also show substantially improved performance in a simple behavioural test involving the discrimination of novel test stimuli, whereas those picked up by the tail show little willingness to explore a test arena and investigate test stimuli. Other laboratories have shown improved glucose tolerance and reduced stress hormone levels when using non-aversive handling methods, or improved responsiveness to sucrose rewards, compared to tail handled mice. I will review the evidence currently available on the responses of mice to different methods for routine handling.

OC11S2

Does mouse handling method have an effect on reproduction of some sensitive inbred lines

Skulj K., Horvat S. and **Pirman Tatiana**
University of Ljubljana Biotechnical Faculty Department of Animal Science, Domzale, Slovenia

Abstract
Routine procedures such as handling or transferring mice from one cage to another can cause a stress response. This is even more important and could be more stressful during pregnancy and at the time of lactation and caring for the litter. In comparison with outbred lines and wild mice, a large fraction of inbred lines

have smaller litters, higher parental age at first litter, longer time from mating to litter etc. The aim of our work was to compare the reproductive results of 2 inbred lines with poor reproduction, FLI (fat) and FHI (lean), in regards to the handling methods. One was the classic method, whereby, mice are picked up by the tail and the other two methods were handling mice with the home cage tunnel or cupping on the open hand. The poor reproductive values are one of the first indicators, if the animals are in stress. In the FHI line the average number of pups per litter was increased from 3.96 to 4.63, the percentage of weaning mice from 87% to 93% and the period between the litters decreased from 40 to 33 days in tail versus the tunnel handling group In the FLI line, those differences were less pronounced, but still the period between the litters decreased from 36 to 29 days. While larger scale study is till warranted, these preliminary results suggest that the handling method could be one of the important factors to improve the reproductive results especially in sensitive inbred lines with poor reproduction.

OC11S3

Tunnel handling of mice: guidance to success

Van Loo Pascalle, Van Leuffen N., Schmitz E. and de Leeuw W.
Animal Welfare Body Utrecht, Utrecht University, Utrecht, Netherlands

Abstract
Hurst and West (1) introduced tunnel handling as a more mouse friendly alternative to tail handling with overwhelmingly convincing results implicating less stress and better research subjects. Under the auspices of the NC3Rs (2), a campaign has been launched to aid technicians and facilities in implementing the tunnel handling technique. Enthused by their approachable tutorials and tips, we investigated the feasibility of implementing the tunnel handling technique in our facility.

Results of our study and subsequent workshops showed that technicians were eager to implement the technique if it would indeed benefit the wellbeing of the mice. There was, however, some scepticism with regard to possible extra time needed for animal care taking, ease of inspection of individual mice and loss of beneficial effects of tunnel handling after invasive techniques, even though results proved otherwise.

We concluded that for successful implementation of tunnel handling, it is crucial to show patience towards untrained mice and let them get acquainted with the tunnel, to appoint trained tunnel handlers to guide novices, and to be alert to correct use of the technique throughout the training process. Furthermore, it is important to involve management from the start and proactively think along on how to narrow time and costs during the implementation process.

We are now implementing the technique in our own facility through workshops and personal training, and offering our training services to technician schools, FELASA C courses and other institutes in the Netherlands.

OC11S4

Teaching of handling refinements

Bugnon Philippe
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Abstract
The initial training of people involved in laboratory animal research (animal caretakers, researchers, ...) is often the first and most important step in their research career to ensure a high quality and reproducibility of their future work. Our task as trainers is to establish basic methods of restraint and applications – a seemingly straightforward task. But the challenges we and the trainees face are manifold: limited time available for training, differences in skills and experience between trainees and the ambitious goals we have as trainers to refine every technique. At the end of the training we certify that the participants are technically able to perform restraining and applications and we have been doing so since 1999 always adapting our training and the techniques along the way. Refinement methods are mentioned in every step of the basic training, but not only as specific changes to the task on hand but also as a methodical approach to any interactions these researchers will ever have with laboratory animals.

OC12S1

What do we want our research animals to be?

Honess Paul
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Abstract
The species used in research have evolved over millions of years. Mostly they are well-adapted to environments where they are found. When removed from their native habitats they are often placed in conditions they are not adapted to and their systems may cease to function adequately to maintain the animal as a true (natural) representative of their species. Where animal use has been fully justified with rigorous harm: benefit analysis and continuous application of the 3Rs, the challenge is to maintain research subjects in a state as close to its natural state as possible to retain the model's validity. We can answer the question: ''What is the nature of (e.g.) a monkey?'' and assess impacts on its natural state. However, we should also ask ''What do we want our animal to be?''. We need them to remain as functionally 'natural' as possible. Together with good veterinary provision animal welfare science contributes to refinement of captive conditions, behavioural management, and husbandry and research procedures towards achieving this goal. This presentation examines how animal welfare science can contribute to producing more valid research models, and stresses the benefits of high welfare standards to build partnerships, improve the quality of science, meet corporate animal welfare standards and reduce reputational risk. Effective animal welfare need not be expensive and yet may add considerably to scientific validity. We should look beyond striving only for cheap research animals and look to improve the quality and translational value of models.

OC12S2

Complex interactions of age and test experience modulate the results of behavioural tests

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Abstract
Recently, a discussion about the reproducibility of results from behavioural phenotyping experiments has emerged. A huge emphasis has therefore been put on the identification of those factors that might limit the reproducibility of behavioural data. As a comprehensive phenotypic characterisation can involve testing of the same animal repeatedly over a specific time period, the aim of the present study was to systematically investigate effects of two potentially confounding variables, age of the animals and test experience. For this purpose, the behaviour of 48 male C57BL/6J mice of two different ages (9 vs. 13 weeks) was assessed in a battery of common behavioural tests measuring anxiety-like and exploratory behaviour (Elevated Plus Maze, Dark-Light test, Open Field test, Novel Cage test). While half of the mice of each age group was naïve to the test battery, the other half had experienced the same tests before. Besides main effects of both age and test experience on anxiety-like and exploratory behaviour, the analysis also revealed profound interactions between these factors. More precisely, an effect of age was apparent in experienced but not in naïve mice. Furthermore, the effect of previous test experience was more pronounced in older than in younger mice. These findings clearly demonstrate that experimental factors, such as age and test experience, can influence behavioural data not just additively, but also in a complex, interactive way. To provide robust and reproducible results, it is thus fundamental to consider such factors systematically in the study design.

OC12S3

Benefits of using automated home-cage systems to study behaviour in group-housed CNS injury models

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Abstract
The translational impact of CNS disease models remains challenged by the limited validity of the behaviour tests used to identify neurological dysfunction. Novel approaches for automatic behaviour monitoring in grouped animals in their home cage provide new paradigms to investigate cognitive and social behaviours in these models.

We studied the use of a non-invasive Home Cage Automatic System (HCA-ActualHCA^{TM}; Actual Analytics Ltd, UK;1) in rat models of Traumatic Brain injury (TBI) and Spinal Cord Injury (SCI), to monitor their behavioural impairments following CNS injury. Automated activity and body temperature recordings were captured along with selected behavioural patterns (aggression, rearing, grooming, feeding and drinking) in adult Sprague-Dawley rats subjected to unilateral closed TBI, thoracic SCI or sham surgery during 12 weeks post-injury, 24h /day up to 3 consecutive days per week. Using the HCA system allowed us to identify specific behaviour patterns such as the display of lower social activity and grooming on SCI animals compared to TBI or sham animals during light and dark phases. TBI animals showed a significantly higher aggression during the acute phase post-injury. No differences in drinking or feeding were detected between the groups. The degree injury severity was confirmed using standard locomotor scoring systems and histological analysis.

The ability to analyse the behaviour of identified single animals within a grouped housed setting represents a major 3Rs benefit, providing a non-intrusive and highly comprehensive experimental tool for the long-term monitoring of intrinsically complex models of CNS injury, with great potential for their translational validity.

OC12S4

Sex specific model of chronic stress

Balog Marta[1], Debeljak Ž.[2], Mandić D.[2], Blažetić S.[3], Labak I.[3], Ivić V.[1], Zjalić M.[1] and Bardak A.[1]
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Abstract
Introduction: Chronic stress (CS) research in rodents is often incomparable because of different protocols used and is also biased because mostly males are used. We developed a protocol of CS in male and female rats validated by serum hormones analysis.
Methods: Male and Female Sprague-Dawley-CR rats were divided in young and old groups. Protocol was performed through 10 weeks. Chronic stress was induced by combining various physical and psychological stressors: disturbance of the circadian rhythm by lights and noise during the night phase, restraint in metal tubes, exposure to cold, forced swimming and rotation of rats in the cages using a laboratory shaker. To validate CS protocol serum was collected from all animal groups at the begining and the end of study. Corticosterone, progesterone and testosterone were analysed by mass spectrometry. Study was approved by Croatian Ministry of Agriculture (602-04/14-08/06).

Results: Corticosterone was increased in young males and old females ($p = 0.036$) upon CS compared to control groups at the end of the study. Comparison of baseline and end measurement showed increase in corticosterone concentration in young males ($p = 0.008$) and old females ($p = 0.009$). Progesterone increased in old females upon CS at the end of the study ($p = 0.018$) while in young CS females progesterone decreased at the end measurement in comparison with control group ($p = 0.03$). Testosterone increased in old CS males compared to control group at the end of the study ($p = 0.012$).

Discussion and conclusion: CS protocol is validated by increased corticosterone in young males and old females. Progesterone could be important in neuroprotection upon CS in old females and testosterone in old males. These results imply gender and age differences in mechanisms of chronic stress response.

Acknowledgement: This study has been funded by Croatian Science Foundation and This study has been funded by European Union through European Regional Development Fund, Operational Programme Competitiveness and Cohesion, grant agreement No. KK.01.1.1.01.0007, CoRE – Neuro.

OC12S5

The importance of sex differences in cognitive behaviour studies

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Abstract
The sexual dimorphism of some brain structures (Ruigrok et al. 2014) is widely studied. During designing and evaluating animal experiments, especially those of cognitive-behavioural types, the consideration of the sex differences is crucial. Some parts of the cortex are heavier in male than in females. During the evolution the females developed higher amygdala activity and a more active functioning of the hippocampus (Jacobs, 1996). The male brain is functioning on a stronger lateralisation. In females, the regional nodes are connected with both hemisphere, in turn, males showed a strong interconnectivity only in case of the cerebellum (Ingalhalikar et al. 2014). The volume of the female amygdala may change after fearful experiences. The oxytocin may improve the spatial memory, too. Koebele et al. (2018) have found, that even the fact of the hysterectomy, had a significant detrimental effect on the execution of complex cognitive task ("hysterectomy-induced memory deficit"). The psychological sex differences are due to the interaction of genes, hormones and (social) learning of brain. The oestrogens have an influence even on the perception of the brain (Tobias, 1965). The microbiome differently influences the concentration of neurotransmitters in the hipocampal serotonergic system in the males and females (Clarke et al. 2012). Several sex differences are demonstrated in the pharmacodynamics, metabolism, pharmacology and toxicology of several chemicals (Schwartz, 2007). As a conclusion, it can be emphasised, that besides the actual standardisations, the sex differences should be considered in the experimental design; the cognitive-behaviour-memory test should be carried out on both sexes.

OC13S1

Pioneering examples from the pharmaceutical sector of disseminating and progressing animal welfare initiatives

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Abstract
The European Federation of Pharmaceutical Industries and Associations (EFPIA) represent the pharmaceutical industry operating in Europe. Through its direct membership of 36 national associations and 40 leading pharmaceutical companies and Partners in Research including CROs, we are involved in a number of initiatives, which affirm the key principles of the 3Rs and support animal welfare. As part of this commitment, the European pharmaceutical industry promotes these initiatives internally and externally.

The presentation will describe the global approach to disseminate and implement animal welfare initiatives. It will give pioneering examples from the EFPIA membership on innovative technologies (Organ-on-a-chip), new housing methods (metabolism cage and group housing) and animal welfare and model refinements (long term non-invasive analgesia, telemetric devices) and methods moving towards replacement. In addition, the presentation will also address how fostering a culture of care has worked as an enabler to progress and develop animal welfare initiatives.

OC13S2

Are sham operations necessary in surgical kidney models?

Atkinson John
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Abstract
Chronic Kidney Disease (CKD) currently consumes around 5% of the NHS budget in the UK. Most forms of CKD progress to End Stage Kidney Failure (ESKF) through progressive fibrosis of the organ. The two most commonly used models of renal fibrosis are the subtotal nephrectomy (SNx) model, and the unilateral ureteral obstruction (UUO) model. The UUO model is a relatively simple model performed by tying the ureter of the left kidney allowing urine backflow and fibrosis like obstructive nephropathy. This model allows no functional data to be collected as the right kidney remains functional. The $5/6^{th}$ Subtotal Nephrectomy model is a hypertension-driven model of CKD. It is characterised by initial tubulointerstitial fibrosis and glomerulosclerosis, leading to renal failure in 90–120 days. Normally, the control animals in these models have a 'Sham' operation. We sought to determine if this surgery was necessary, with functional and histological comparisons between Sham animals and Non-Operated animals, and comparisons between these and the contralateral right kidney which remains in the UUO model. For the SNx model, we found

no significant difference between a sham-operated animal and a non-operated animal in terms of function (serum creatinine, creatinine clearance, albuminuria, blood pressure) or histology (Masson's Trichrome or Sirius Red staining). In the UUO model we found no difference in histology between a sham-operated animal and a non-operated animal. We feel, therefore, that in the interests of animal welfare and the 3Rs, that exposing animals to the stress of sham surgery is unnecessary.

OC13S3

Handling and training for less stressed laboratory rodents

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Abstract
Since 2015, we have actively worked to reduce the stress levels in laboratory rodents to prepare them for experimental procedures in our toxicological studies. This work has been acknowledged and rewarded with the EPAA Refinement Prize 2017 for our work methods leading to calmer rats and mice during experimental procedures, and the FELASA 40 Years Anniversary Technical Award for our initiatives towards the furtherance of animal technology. Our presentation will describe how we work with 1) housing and enrichment, 2) handling and training, and 3) experimental procedures. We will also present how the stress reduction in our animals allows for lowering the amount of anaesthetics needed to achieve optimal sedation.

OC13S4

The Veterinary Refinement Initiative – Strategic implementation of refinements in a UK academic institution

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Abstract
Refinement of scientific procedures is a legal and ethical requirement under various legislation, including the European Directive 2010/63/EU and Animals (Scientific Procedures) Act 1986. This aims to minimise the adverse effects and maximise the scientific benefit from scientific procedures carried out on protected animals.

However, in the academic setting effective implementation of refinements can be impeded by factors such as numerous and independent research groups which make effective communication and dissemination of best practise challenging. Moreover, research tends to focus on specific organ/ systems and scientific endpoints, with the impact on animal health and welfare possibly being a secondary consideration. In addition, reference data are based on well-established published methodology, thus requiring time-consuming and potentially costly validation of novel, 'more refined' techniques.

Therefore, despite considerable time and effort, implementation of refinements tend to be short-lived and wider uptake may remain low. The 'veterinary refinement initiative' was developed as a two tier support strategy combining regular, structured and systematic protocol review with support structures such as training workshops and regular 'road-show' lectures on relevant topics such as aseptic surgical technique. Well planned and written method sheets to facilitate regular protocol review, staff training and optimise planning are encouraged. This strategic and novel approach of a 'veterinary refinement initiative' has been positively received by the academic research community and instrumental in facilitating effective promotion and dissemination of refinements to benefit animal welfare and science alike.

OD1S1

Quality Assurance in sourcing of ruminants & pigs for research use

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Abstract
Ensuring the provision of suitable quality level animals is one of the keystones to a responsible and efficient research, as well as to the compliance to animal welfare and 3Rs obligations.

Sourcing of agriculture species, not available from ''purpose bred'' colonies, is a complex and sensitive issue embracing research quality-related, 3Rs implementation and personnel safety. Ensuring a relevant quality level requires defining and implementing an appropriate Quality process.

The Quality Assurance management process presented includes the following steps:

- Setting the scientific and veterinary specifications for the expected products and/or services,
- Formalizing a comprehensive Quality Technical Agreement (QTA) defining these specifications as well as reciprocal expectations and responsibilities, applicable standard and regulatory requirements,
- Defining roles and responsibilities for both parties, including the change control process and for QTA management,
- Stipulating the conduct of ''Periodic Reviews'' and Audits (initial, periodic and ''for cause'') using the QTA as reference document,
- Reviewing the QTA, with all competent representatives from each party (scientific, technical, quality, purchasing, legal),
- Finalizing and signing the QTA by authorized from each party

The different sections of this document will be presented, briefly commented and illustrated by some selected examples.

OD1S2

Our journey to SPF sheep

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Abstract
Healthy animals are prerequisite condition for successful preclinical studies. Health problems encountered during the study may bias research results, increase the burden or even lead to the exclusion of the affected animal. Furthermore, certain diseases also endanger persons taking care of the animals. To avoid this, rodents and rabbits bred for research purposes in specialized facilities are Specific-Pathogen Free (SPF) according to FELASA (Federation of European Laboratory Animal Science Associations) health monitoring recommendations. In contrast, sheep are very seldom bred on purpose for research and are usually purchased from local farms. Even though FELASA recommendations for screening of the most relevant pathogens (bacteria, viruses and parasites) also exist for sheep, neither the farmer breeders, nor the very few specialized breeders, report following them, nor breed SPF sheep.

In 2017 we have successfully managed to set the premises of the first European SPF flock, according to the FELASA Recommendations. We have performed Caesarean-sections and obtained SPF lambs free of, inter alia, *Chlamydia* spp., *Coxiella burnetii, Corynebacterium pseudotuberculosis, Mycobacterium paratuberculosis, Salmonella spp., Leptospira spp.*, Small ruminant lentiviruses, Border Disease Virus.

We are aiming to have a self-sustained flock of SPF Sheep, which will then only require Caesarean sections to bring in new genetics, all other births will be natural within the developed SPF flock. In future, these sheep will be available for researchers.

Here, we report on the challenges, failures and successes encountered and ahead of us during our journey to SPF sheep.

OD1S3

Example of small ruminants' health monitoring

Ferrara Fabienne, Dühlmeier R. and Füner J.
Pet and Farm Animal research department, Preclinics GmbH, Potsdam and Campus Eystrup, Germany

Abstract
Introduction: Small ruminants (SM) are of high importance in bioscientific research and frequently used as a model for human research as well as for agricultural or veterinary studies. At the Preclinic Research Farm, SM are utilised for collection of fresh blood or biological products. The company also provides customers with a well-controlled source of SM for use in research trials. Working with SM in the field of research or production of medicinal diagnostic products is challenging, as SM are usually obtained directly from livestock production, which can lead to a reduced animal health and hygienic status.
Methods and Results: Practical health- and hygienic monitoring programs are continuously refined at the Preclinic Research Farm to ensure a well-controlled supply of small animals to our customers. Establishment of these methods allows the production of safe fresh blood or biological products. To establish specific pathogen-free SM, animals were acquired from carefully selected farms and underwent a prolonged quarantine period with repeated testing. To date, Preclinic Research Farm has established methods to ensure a 'basic control' level for SM. Due to the different demands of various research fields it is necessary to establish specific monitoring programs to gain SPF-SM.
Conclusion: To ensure data validity and safety of fresh blood or biological products, it is relevant to use well-controlled SM. Therefore, it is necessary to establish SPF-SM by using practical health and hygienic monitoring programs. At Preclinic Research Farm we are continuously refine such programs to gain SPF-SM cohorts.

OD1S4

Importance of post-weaning stress and its interaction with the intestinal barrier function

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Abstract
Weaning is a critical period in which homeostasis can be threatened by psychosocial and environmental factors. Improving the adaptation of piglets in the weaning period is crucial to reduce gastrointestinal disorders that may cause mortality and pathological complications. Our aim was to study the interaction between husbandry and intestinal barrier function in the weaning period. The experiment included 22 Danbred breed male piglets weaned at 26 $\pm$1 day. Upon born, animals were assigned to one of the 2 experimental groups: control or enriched. The control group followed the conventional husbandry farm conditions. The enriched group was provided with enrichment materials, such as toys, in the pens; animals from the enriched group were also allowed to have timely contacts with piglets from other litters in the pre- weaning period. 3-4 days after weaning, colonic fresh samples were obtained and mounted on Ussing chambers to measure the integrity of the intestinal barrier and the ion transport of the colon. Compared to control piglets, enriched piglets exhibited reduced baseline electrical values (short-circuit current (I_{sc}) and transepithelial voltage (PD)). None significant differences were observed in the conductance (G) and the paracellular permeability between groups. Although none differences were observed in the permeability between groups, the increased electrical values observed in the none-enriched group indicate a higher degree of active ionic transport. Electrogenic secretion is a marker of diarrhoea, indicating that enrichment is reducing the intestinal secretion and contributing to a less incidence of diarrhoea in just weaned piglets.

OD2S1

Pain Management in Large Animal Translational Research Models; Problems

Clutton Eddie
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Abstract
Pain management (recognition, quantification and treatment) in all laboratory animal species is a societal expectation, and a legal, moral, practical and scientific requirement. However, in comparison to: a) small laboratory species, i.e. rodents, undergoing similar procedures; and b) large animals undergoing noxious husbandry procedures, e.g., castration, under farm conditions, pain management in large laboratory animals is infrequently and, or poorly reported. Therefore, the increasing use of pigs and sheep in translational research runs the risk of greater suffering. This is counter to humane experimental technique (the 3Rs) and may affect scientific outcomes. Effective pain management represents experimental refinement and reduces the physiological noise arising from nocistimulation. Producing noise-free data increases study power and reduces the number of animals required to achieve scientific outcomes. Improving pain management in large laboratory animals is complicated because: 1) contemporary veterinary anaesthetic practice promotes pre-emptive analgesia a technique which reduces the likelihood of overt pain signs being manifest; 2) severity level and humane end-point imposition by the legislature and ethical review processes mandates the use of ''rescue'' analgesia, further limiting evaluators' familiarity with pain behaviours. Large laboratory animal pain management could be improved by: 1) pain studies in sheep and pigs; 2) a more aggressive adherence to the ARRIVE guidelines; 3) improved communication between those interested in the subject.

OD2S2

Anaesthesia and analgesia protocol for a pig thoracotomy model

Bergadano Alessandra
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Abstract
What is special in the pig: Very stress sensitive (hyperthermia, arrhythmias), not very handy, difficult IV access and intubation, prone to malignant hyperthermia, pauci-symptomatic... preys...

What is special in the procedure: Ventilation is required. Severe pain is expected. What is the model for? Bias of anesthesia and analgesia on the model? Special challenges (fluids, blood) of the model?

What is the key to successful anesthesia: A balanced multimodal anesthesia and analgesia protocol (PIVA) with emphasis on loco-regional anesthetic techniques: intercostal block vs epidural analgesia and soaking catheter. Neuromuscular relaxants and ventilation (IPPV) is a must. Check the T° trend: be cool. What makes the anesthesist happy: All the fancy monitoring: TOF, spirometry, invasive blood pressure and modern ventilators. What makes the pig happy: An empty bladder (urinary catheter and outflow control), multimodal analgesia with NSAIDs buprenorphine and bupivacaine in the soaking catheter. Monitor SpO2 at recovery until fully deambulatory and have O2 and thorax drain kit available. Dedicated nursing scoring my daily condition 3 times a day and Goodies are welcome. The wag my tail scale. What is different in macaques: Easy intubation but beware of regurgitation. Very hypotensive with inhalation anesthesia, especially isoflurane: (invasive) blood pressure measurement and vasoactive support. Small= hypothermia! Post-epidural locomotor impairment to be avoided. No catheter in place without a jacket ... fentanyl patch or long-term analgesics. Nice cozy O2 and T° enriched cage for recovery. Pain assessment quite challenging as TLC. Keep in mind the zoonotic potential and wear adequate protective equipment.

OD2S3

Anaesthesia and analgesia for pregnant sheep models of preterm birth

Musk Gabrielle
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Abstract
Pregnant sheep are commonly used as models for investigating the causes and consequences of preterm birth. The considerations for this model include the physiology of pregnancy, the gestational stage, pain assessment, the risk of pregnancy toxaemia and the safety and efficacy of analgesic drugs. The theoretical physiological alterations of pregnancy do not usually present significant clinical challenges, but as gestation advances the side effects of mechanical ventilation on uterine perfusion may be exacerbated. Pain assessment in sheep is difficult, especially in an animal facility environment. The identification of mild to moderate pain is particularly challenging so an approach, which incorporates composite behavioural and physiological observations is important. Pregnant sheep are at risk of pregnant toxaemia if there is an interruption to their feed intake. Careful introduction and acclimatisation procedures prior to surgery will mitigate this risk as will frequent clinical observation to ensure their feed intake is adequate. The safety and efficacy of analgesia drugs for pregnant ewes and their fetuses is controversial and lacking in evidence. A multimodal, preventive analgesia regime that is administered for an appropriate duration of time after surgery is essential. Opioids can be used in pregnant sheep, local anaesthesia is indicated in this model and ketamine can be incorporated into the anaesthetic protocol to enhance the analgesia. The use of non-steroidal anti-inflammatory drugs is more difficult to promote, as the risk of premature partial or complete closure of the fetal ductus arteriosus is a risk that must be avoided.

OD3S1

FELASA Working Group on Farm Animals: Outcome

Berset Corina[1,2], **Ferrara Fabienne**[3,4], Caristo M.E.[5], Hardy P.[6], Oropeza-Moe M.[7], Sossidou E.[8] and Waters R.[9]
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[9]*The Pirbright Institute, Pirbright, UK*

Abstract
The FELASA Working Group on Farm Animals has been created in 2017, after a joint initiative of the representatives of three networks (from Germany, UK and Switzerland) for farm animals in biomedical research. These networks had identified a knowledge and reporting gap regarding the current practices involving health and hygienic monitoring of livestock species used for experimental purposes. Their representatives initially proposed to organize one day of sessions on farm animals at the FELASA Congress 2019. Subsequently, FELASA has nominated 7 representatives of national societies for laboratory animal science – SGV(SLAS), GV-SOLAS, AISAL, AFSTAL, Scand-LAS, HSBLAS and LASA – as members of the Working Group, based on their previous experience with farm animals.

The Working Group's other tasks were to identify relevant pathogens in livestock production and experimental animal facilities in respect to different scientific procedures, define standard criteria of animal acquisition to obtain more pre-defined animals, review existing recommendations and practice in farm animal health and hygienic monitoring and propose new, practical recommendations of health and hygienic monitoring strategies for ruminants and pigs.

The Working Group has successfully reached its goals. The FELASA 2019 comprises four sessions on farm animals used for research purposes, while new and practical recommendations for animal health and hygienic control with ruminants and pigs have been developed. After a formal review by FELASA, these new recommendations will be presented and discussed during an informal review with the audience.

OD3S2

Questionnaires for farm animals users in biomedical research

Ferrara Fabienne[1], **Berset Corina**[2], Jeuthe S.[3], Schmidt T.[4], Pobloth A.[5], Lanker U.[6] and Zeiter S.[6]
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Abstract
Pigs and ruminants used for bioscientific purposes are usually obtained directly from livestock production, leading to uncontrolled and insufficiently reported health and hygienic status. Therefore we aimed to evaluate the status quo of the implementation of animal health and hygienic monitoring programs, by electronically distributing 2 questionnaires (Q1 and Q2). Q1 was an extended survey for farm animal users in biomedical research in Germany, Austria and Switzerland. Q2 was conducted among European sheep users, with emphasis on animal selection criteria and practical issues. For Q1, 29 participants were included in the data analysis. The answers confirmed that measures of animal health- and hygienic control are less standardized and more basic than those defined for rodents. Access control and the changing of clothes and shoes was performed in all facilities, but a strict barrier system was only implemented for pig housing. Overall, a routine quarantine period was not maintained within the most facilities, which is may be related to the lack of space within housing facilities. More than 50% of the 84 respondents to Q2 had encountered problems in their sheep not related to the experimental protocol and did not have a health monitoring program. The main criteria for choosing a sheep supplier were the animals' health status, followed by their availability, the trust and experience in the sheep provider and the animals' uniformity. The results of both questionnaires obviously underline the need to develop relevant and practical guidelines for animal health and hygienic status monitoring and control in research facilities.

OD4S1

Housing and husbandry of zebrafish: The contribution of the FELASA Working Group

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Abstract
Zebrafish (*Danio rerio*) are increasingly used as vertebrate organisms to model key questions raised in basic and applied research including, but not limited to biomedicine, toxicology, environmental

science, biotechnology and aquaculture. In Europe, zebrafish research is covered by the European Directive 2010/63. The Annex 3 of the Directive, on ''Requirements for establishments and for the care and accommodation of animals'', in the Part B does provide only general requirements. Currently, numerous and varying husbandry procedures are available from the different laboratories/facilities housing zebrafish. However, standardisation protocols, providing ranges of husbandry parameters, are necessary to ensure the animal welfare as well as reproducibility of experimental procedures. A joint working group (WG) on zebrafish housing and husbandry recommendations, composed of members of the European Society for Fish Models in Biology and Medicine (EUFishBioMed) and of the Federation of Laboratory Animal Science Associations (FELASA) has been given a mandate to address a FELASA list of parameters, "Terms of Reference", and give recommendations for related guidelines. These recommendations for laboratory zebrafish care and use can help the further implementation and the fulfilment of Annex 3, Part B of EU Directive 2010/63, both concerning the housing and care of experimental animals.

OD4S2

Fishes are not wet mice: How to establish a fish facility fulfilling welfare requirements

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Abstract

Working with the Zebrafish *Danio rerio* as an animal model was initiated at the Ecole Polytechnique Fédérale de Lausanne in 2017, to support the development of new paradigms. To this end, a new fishroom was built to meet the highest standards in cutting-edge research, husbandry and animal welfare. Special attention was given to the design of the water supply system, to ensure the highest quality of water delivered daily into the tanks. Similarly, the facility was designed in such a way that sentinel animals tanks and quarantine tanks could be integrated into the main water circuitry. The food delivery system was tailored to the physiological needs of zebrafish, relying on recent developments of robotic feeding devices. Those choices proved to be sound, as shown by the survival rate, the breeding efficiency and growth rates of the fishes. Once established, our facility was accredited by the Cantonal Veterinarian Authorities, according to the requirements of the Swiss Animal Welfare Act and ordinances on Animal Protection and Experimentation. During this process we realized that fishes were often considered as ''wet mice'' due to the lack of species-specific guidelines. The Authorities paid close attention to the enrichment of the tanks, to the anesthesia and euthanasia methods, and to the characterization of the transgenic lines exhibiting a pathological phenotype. These regulatory requirements revealed the lack of deep scientific knowledge in these fields, and the needs for more precise guidelines, which should be addressed by the Zebrafishes research community in a coordinated way with the authorities.

OD4S3

Characterization of zebrafish post-embryonic development under defined husbandry conditions

Borges Ana

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Abstract

Zebrafish embryonic developmental has been well characterized over the past decades, and reference tables are widely available. During this early period, the maintenance of these embryos in the laboratory setting is simple and highly reproducible. In order to obtain synchronized and healthy embryos/larvae, only a few variables need to be controlled, such as the embryo medium quality and temperature. However, as they undergo larval development and metamorphosis, and yolk reserves are depleted, environmental variables become more prominent, thus greatly affecting growth and developmental rates. The most commonly used unit to characterize larval stages found in the literature is *days post fertilization*, or *dpf*, however, this translates poorly to actual size or developmental status of individuals. This may lead to low reproducibility, as different rearing conditions, for the same period of time, may produce different outcomes.

In order to further investigate post-embryonic survival and growth under specific environmental conditions, we have been rearing larvae batches in defined husbandry conditions. We have evaluated combinations of the following parameters: water quality management, feed type (rotifers vs processed feeds), animal density, water/media composition and temperature. For each set we have assessed growth and survival rates.

Our goal is to contribute to increased reproducibility by providing a reference developmental table of zebrafish larvae under specific rearing conditions. So that whenever a specific stage of development is required for a given experiment, the researcher knows what the required husbandry conditions are.

OD4S4

Challenges in the maintenance and breeding of wild zebrafish in a laboratory environment

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Abstract
Zebrafish, *Danio rerio*, is a widely used model organism for a range of research topics. While the majority of this work has been conducted using domesticated strains, there is an emerging interest in the use of wild-origin zebrafish in research. The behaviour and physiology of wild zebrafish is vastly different from domesticated strains and so traditional breeding and maintenance practices are generally unsuitable. Here we outline three broad challenges and solutions that have emerged during our recent work with a population of wild zebrafish. First, the wild fish appeared agitated in the laboratory environment and so a step-by-step re-housing protocol was required to acclimate wild fish to being kept in smaller tanks. The use of enrichment (substrate and plants) aided in this process. Second, wild-origin zebrafish were not amenable to breeding practices which are generally applied to domesticated strains. However, placing domesticated fish in neighbouring spawning tanks triggered courting behaviour in wild fish and resulted in a six-fold increase in the number of egg laying wild couples. The final challenge was managing pathogens present among the wild fish. Histological analysis of wild fish revealed the presence of mycobacteriosis. Here we present data on the efficacy (measured by egg survival rate) of different methods for egg disinfection (NaClO; PVP-I; KMnO4) at a range of concentrations and soak times, to prevent transmission of *Mycobacterium spp.* to the next generation via waterborne exposure. These results will aid other facilities in overcoming the challenges encountered while working with either wild zebrafish or domesticated lines.

OD5S1

Cephalopods and the Directive 2010/63/EU: Species-specific minimum requirements for care and accommodation

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Abstract
Cephalopod molluscs represent a diversified set of marine invertebrate species (approximately 800) characterized by different habits, life history and physiological adaptations. From January 1st 2013 all ''live cephalopods'' are included in the list of regulated species by the Directive 2010/63/EU. General guidance (Section A) and species-specific minimum standards (Section B) of care and accommodation of animals covered by the Directive are provided in Annex III. These recommendations are restricted to vertebrates and no information for any cephalopod species is available. 'Guidelines for Care and Welfare of Cephalopods' (https://www.ncbi.nlm.nih.gov/pubmed/26354955) are now available and include a general set of recommendations for the care and welfare requirements of these animals. Recently, the COST Action FA1301 'Cephs*In*Action' attempted to harmonize research on cephalopod biology and standardize approaches. Here we illustrate the outcome of a consensus process involving over 40 scientists and aimed to facilitate uniform recommendations for minimal requirements for the care and accommodation for the most commonly utilized cephalopod species. After an accurate overview of scientific literature we compiled a ''cephalopod Welfare table'' based on over 600 different published works. In the table information on water supply, environmental requirements, accommodation and care, housing, diet, enrichment is provided for: *Nautilus pompilius*, *Sepia officinalis*, *Sepia pharaonis*, *Euprymna scolopes*, *Sepioteuthis lessoniana*, *Loligo vulgaris*, *Octopus vulgaris*, *Octopus bimaculoides*and *Octopus maya*. The 'cephalopod Welfare Table' is considered as a standardization and harmonization tool and is at the basis of suggested recommendation for mandate minima as applicable to live cephalopods in scientific research.

OD5S2

Description of husbandry and health programs of a multi-species aquatic facility

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Abstract
Aquatic animal models have greatly contributed to experimental biology, embryology and stem cells research. Both invertebrate and vertebrate aquatic species have been used since the 18th century to uncover basic aspects of biology.

Our facility works with 3 aquatic species: the frog *Xenopus laevis*, a classical model to study embryology, cell biology and biochemistry; the zebrafish (*Danio rerio*), a prominent fish model for studying embryology, organ regeneration, human disease, and toxicology amongst other areas; and the african turquoise killifish (*Nothobranchius furzeri*), a model organism to study aging and associated diseases.

Research conducted at the Instituto Gulbenkian de Ciência (IGC), an institution with a long tradition of organism-centered research, integrates not only rodents and flies but also the three above cited aquatic model organisms. The co-existence of multiple aquatic species in a vivarium poses challenges at many levels, namely biosafety and health control, facility design, and specialized human resources.

In this talk we will describe the strategies we have been developing over the years to overcome some challenges, namely:

- facility design
- facility routines
- human resources
- implementation of a comprehensive health program adapted to the different animal species
- biosecurity risk assessment, disinfection protocols and circulation of animals, personnel and material
- mitigation of pathogen contamination between species

OD5S3

Breeding and maintenance of Nothobranchius furzeri: A story about constant improvement during fourteen-year experience

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Abstract
The Leibniz Institute on Aging Jena has a fourteen-year-long history of breeding and maintenance of *Nothobranchius furzeri* under laboratory conditions. The fish facility has played a key role for scientists in establishing of *N. furzeri* as successful model organism in aging research. We house more than 2700 fish, available for experiments, and more than 1700 fish exclusively in the breeding unit.

Especially in the last three years, our goals were the optimization and standardization of operating procedures in the facility to provide a solid program of keeping different strains, particularly targeted on generation of genetically modified organisms. The maintenance of certain strains can be demanding; the strain GRZ, for example, shows a specific phenotype when inbreeding depression occurs. The genetic pool of each stain can be compromised if not monitored by a reliable embryo-management. We established a breeding plan of our colony to reduce the generation of animals with an undesired phenotype to a minimum and to avoid lifespan alterations of different strains.

For our colony management, a solid and comprehensive health monitoring is paramount. It consists in daily observation with standardized criteria by trained personnel, regular screening of specific pathogens, and a rigorous hygiene plan. The program bases on the risk assessment that takes into account scientific requirements, logistic and infrastructure characteristics, as well as the coexistence with a *D. rerio* facility. The bio-exclusion design of our health and hygiene plan aims to prevent cross infections and to recognize early outbreaks, to avoid dramatic loss of the colony.

OD5S4

Fishing in the Dark – Working with cavefish (Astyanax mexicanus)

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Abstract
With recent advances in molecular technologies accompanied by the reduction in both time and cost to sequence whole genomes, researchers are turning to new model organisms to answer fundamental questions. New species not only allow original avenues of research, but also allow for comparison with existing model organisms.

At the Stowers Institute we have worked with multiple emerging model organisms over the past decade and this talk will focus on cavefish. Cavefish provide a great opportunity to interrogate developmental and evolutionary genetics due to the existence of both surface and cave morphs. This talk will provide fascinating insights into the species, along with a look into the challenges of setting up these animals in a research facility environment, including housing, husbandry, and colony management.

OD6S1

Zebrafish health monitoring: Recommendations from the FELASA-AALAS Working Group

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Abstract
When exchanging fish for research, facilities open their doors to a potential hazard as incoming fish may carry relevant pathogens. To help mitigate that risk, FELASA and AALAS established a joint working group, with three representatives of each association, to develop recommendations on how to define the health status of a zebrafish colony, how to report the screening data, and how to triage imports according to a facility's specificity.

The working group started with a survey on current practices and some analysis of estimated prevalence. Then it defined the list of relevant pathogens and the screening pattern (frequency, type, and number of samples and assays) for routine health monitoring (based on hypothesis of higher prevalence of pathogens). Lastly, a reporting template for historical data and facility description allowing for biosecurity risk assessment was developed.

Some scenarios will be used to illustrate how various types of facilities can adopt and adapt the recommendations. This leads to the question of reliable barriers for different aquatic systems; specifically how to isolate a quarantine area – a requirement for Specific Pathogen Free (SPF) status. Screening patterns for SPF status are then described, based on a hypothesis of low prevalence of specific pathogens, requiring a higher number of samples. For all screening patterns, environmental testing is considered as it can be a useful adjunct to fish sampling.

OD6S2

Combination of direct and environmental PCR for analysis of diseased zebrafish in recirculating systems

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Abstract
Introduction: Reliable detection of unwanted organisms is essential for meaningful health monitoring in experimental fish facilities. Recently, the use of environmental samples from static tanks, like water, detritus or swab samples proved to be a useful method to complement analysis of sentinel fish. In this study, we combined PCR of individual diseased zebrafish with environmental PCR of water and detritus to complement our health monitoring program in recirculating systems.
Materials and Methods: Over a period of 6 months fish showing clinical signs of illness housed in 7 different recirculating water systems were collected and euthanized using overdosed tricaine methanesulfonate. 25 mL of tank bottom detritus were added to 1 L of water from affected tanks and passed through a sterile 0,2 µm filter under vacuum. Frozen filter membranes and fish were sent to a diagnostic laboratory for real-time PCR analysis.
Results: Various pathogens, including different species of mycobacteria, microsporidia and nematodes were detected. Direct fish PCR was superior to environmental PCR for the detection of *Pseudoloma neurophilia* and *Myxidium streisingeri*, but failed in most cases to detect *Mycobacteria spp.* detected readily by environmental samples. *Pseudocapillaria tomentosa* infection was detected by both methods.
Discussion and Conclusions: As demonstrated, neither analysis of diseased fish nor of environmental samples alone allow reliable detection of different pathogens using real-time PCR. A combination of both methods has great potential to become a useful tool to improve health monitoring surveillance programs of fish facilities.

OD6S3

Overview of AKKAB and next target: Recommendations and guidelines for cleaning aquatic housing systems

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Abstract
For more than 12 years the German Working Group for Cage Processing ''AK KAB'' (Arbeitskreis Käfigaufbereitung) has been working on summarizing and publishing state of the art recommendations and guidelines for cleaning rodent cages in a proper way. AK KAB already published 5 editions of their so called ''orange booklet'' in both German and English, either available in printed or digital format (the latter available from the FELASA-website).

Besides rodents as main species in many laboratory animal facilities around the globe, more and more fish or frogs are being used for research as well.

To address the specific needs of aquatic users, the AK KAB recently founded a new working group, consisting of experts for cleaning aquatic equipment. This group inside the AK KAB has now been working for one and a half years, putting together important specific aspects of aquatics housing systems inside biomedical research laboratories. The ultimate goal is to provide recommendations and guidelines for processing aquatics housing systems in a proper way.

The group of experts will present a first structure and initial contents of this new planned ''Fish Booklet''. The idea of this presentation is not to present a final edition of the fish booklet, but to get feedback from the interested audience regarding the planned contents in order to understand what may still be missing or needs more details. The plan is to publish and present the final new booklet in 2020, ready in time for the 15th International Zebrafish Conference.

OD7S1

Update from the FELASA working group on methods of humane killing of laboratory fish

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[3]*University of Copenhagen Faculty of Health and Medical Sciences, Copenhagen, Denmark*
[4]*Electro Fishing Services Ltd, Donaghadee, United Kingdom*
[5]*Joint Production System Ltd, Potters Bar, United Kingdom*
[6]*Parc de Recerca Biomèdica de Barcelona – PRBB, Barcelona, Spain*

Abstract
The FELASA working group on methods of Humane killing of laboratory fish aims at proposing good practice guidance on all relevant chemical and physical methods of euthanasia, whether evoked in Eu. Dir. 2010/63, including electrical stunning, or other methods like hypothermal shock.

The work started with a survey on current practice in order to identify potential issues to address like the lack of established protocol to complete killing of fry. Another major topic of discussion is the definition of humane killing and which requirements should be fulfilled for the anaesthesia process to be deemed acceptable. In order to answer these key questions, the work was divided in several streams; often using zebrafish as an example since it is a common and easily accessible lab fish species.

Literature reviews supported the development of recommendations on how hypothermal shock, i.e. sudden immersion in chilled water, may be used to euthanize zebrafish size fish according to their stage of development. For overdose of anaesthetics, the recommendations are based on experimental studies screening euthanasic solutions against their ability to induce a fast death (monitor time required for all fish in the euthanasic solution to lose the assessed reflexes) with minimal distress (assessed by scoring signs of discomfort).

The working group has not finished developing the recommendations and a discussion on the main conclusions to date will be useful.

OD7S2

Sedation and anesthesia in research fish. Brief review and discussion

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Abstract
This presentation will briefly review and discuss teleost and elasmobranch sedation and anesthesia methods (physical and chemical), anesthesia depth staging, monitorization and the common and recent anesthetics used in research fish.

Physical methods include electronarcosis, mainly used for capture of wild fish, and tonic immobility in elasmobranchs. The appropriateness of tonic immobility in elasmobranch research, namely in morphometric evaluations, ultrasonographic and even in laparotomic surgery, will be discussed. Hypothermia can be improperly considered a physical method of anesthesia. It only immobilizes the fish with no loss of consciousness or analgesia so it can't induce anesthesia.

Chemical methods include inhalation and parental (injectable) administration, being the first one the more frequent method. The commonest inhalation anesthetics used in fish research is the MS222 followed by others like benzocaine, clove oil, eugenol, isoeugenol and 2-phenoxyethanol. In zebrafish, the more used anesthetic is also MS222. Due to eventual relevant side effects and an apparently low safety margin there are recent publications trying to find alternatives to this drug (e.g. metomidate hydrochloride, etomidate, lidocaine hydrochloride, propofol, ketamine and isoflurane). This presentation will discuss these anesthetic options in zebrafish, the described shortcomings of MS222, and how can some of them be mitigated.

OD7S3

An overview of anaesthesia in Cephalopods: Challenges towards a standardised protocol

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Abstract
We review over 100 years of studies where Cephalopods have been used as experimental animals in procedures requiring anaesthesia. Substances used, and reported effects on animals in terms of induction, condition during anaesthesia, and recovery are considered, whenever original data are available. The aim is to propose a standardized protocol, specific for each of the most frequently utilized cephalopod species. The focus is on a conservative selection of substances, the physiology of each species and the applicability of criteria developed for anaesthesia in other laboratory animals. Finally, a pipeline is proposed for selecting anaesthetics to be applied in procedures where minimally-invasive or extended surgery is required. We are soliciting a coordinated effort towards the final goal of developing standardized procedures for anaesthesia of Cephalopods based on their physiological, biological and welfare requirements.

OD8S1

Our team journey towards a safe and humane transport of laboratory animals

Dudoignon Nicolas
FELASA-AALAS working group on the transport of laboratory animals

Abstract
Due to the globalization of research and of animal models' sources, there is an increase of exchange of laboratory animals, between specialized breeders and research institutions, between research groups, and also in the context of multi-site projects.

Supply chain is a critical step, and any means of transport should be in custody. Laboratory animals move from one controlled and monitored area to another and it is not unusual that four or five companies are involved between the breeding colony and the research center, and that three or four pieces of regulations are referred. Nevertheless, thanks to the professionals involved, very few incidents are reported.

Although the transport of animals is highly regulated, there are differences between American and European countries, and even within EU, and with other parts of the world. Those are complex regulations and not dedicated to laboratory animals. In some cases, the regulation is not appropriate to laboratory animals.

An FELASA-AALAS working group on the transport of laboratory animals was established to set up recommendations related to good transport practices. Although, it was agreed that the whole and complex transport framework could not be synthetized in a single document, the group aims at providing the research community with a user-friendly tool where most relevant references would be made available.

This session will include *i)* an update of the working group activities, *ii)* considerations about animal transportation across EU (including post-Brexit consequences if applicable…), and iii) specific aspects for a set of relevant species.

OD8S3

International harmonization of zebrafish shipping regulations is essential for fish survival and welfare

Varga Zoltan
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Abstract
Scientific efficiency, reproducibility, and progress depend significantly on collaboration between scientists and liberal exchange of ideas and resources. Arguably, the most important and vulnerable resources are the animals entrusted to our care. The Zebrafish International Resource Center is a central repository for wild-type, mutant, and transgenic zebrafish lines, with considerable experience and expertise in domestic and international shipments of zebrafish[1]. The ZIRC also specializes in zebrafish welfare, health, and husbandry and provides veterinary fish diagnostic services partnering with OVDL[2]. To date, ZIRC has shipped over a million fish (almost 10,000 shipments, virtually 100% shipping success). Keys to successful shipments are standardized packaging and controlled shipping conditions, including animal density, water, air, and temperature. Knowledge of international import requirements is also a prerequisite for shipping success. Expedient shipping and transit to the destination facility guarantee a minimum of harmful waste product build-up in fish water and minimize exposure of packages to detrimental environmental influences.

In recent years, the exchange of laboratory fish has become significantly more complex due to increased regulatory burdens for animal imports that frequently vary between countries. In spite of a common EU directive for the transport of live animals, regional differences in interpretation at points of import threaten to delay shipments between laboratories. To prevent animal death, distress, or discomfort in transit, and to maintain adherence to national and international animal transport regulations, ZIRC provides a successful method for international shipment of laboratory zebrafish and supports harmonization of laboratory fish exchange in Europe.

OD8S4

FELASA Working Group on capture and transport of cephalopods "FELASA WG-CTCephs"

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[3]*SECAL – Sociedad Española para las Ciencias del Animal de Laboratorio, Las Palmas de Gran Canaria, Spain*
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[5]*FIshery, Lisbon, Portugal*
[6]*Flying Sharks, Horta, Portugal*
[7]*Tecniplast, Buguggiate (Va), Italy*
[8]*Association for Cephalopod Research, Naples, Italy*

Abstract
Cephalopods were the first class of invertebrates to be regulated in the EU (Directive 2010/63/EU) for their use for scientific purposes. Either capture in the wild (still needed due to specific challenges in captive breeding) or sharing of individuals between different laboratories require transport. These can interfere with the welfare of cephalopods and, therefore, with the validity and reproducibility of experiments and should be only accomplished by competent persons. Council Recommendation 2007/526/EC of 18/06/2007 does not include specific reference to cephalopods, and general sections do not provide possible guidance.

WG-CTCephs aims to address good practices and related challenges to develop recommendations for standardization of methods to be used not only by researchers, but also by fishermen and any others who capture and/or transport live cephalopods.

WG-CTCephs focuses on 4 main sections:

1. Regulation and documentation for transport and capture: since cephalopods transport is not exclusively performed within the EU, international Codes and Guidelines developed by Independent Organizations were considered.
2. Review of existing capture methods for collection of live cephalopods in the wild and how to maximize welfare during this critical action.
3. Review of existing transport methods at local (short term), national and international level and its impact on the health of animals.
4. Needs of Education and Training programs for collectors, shippers and transporters.

Different backgrounds of WG members (scientists, fisherman, veterinarian, industry) helped addressing all main topics and to exchange views on how to overcome the existing challenges.

OD9S1

Implementation of a culture of care and international recognition in the academic environment

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Abstract
There is plenty of excellent information and advice available about a Culture of Care in laboratory animal science that can assist you in developing your own programme. This presentation will show how a Culture of Care has been developed in an academic environment that has recently been AAALAC accredited. It details what challenges we faced, how these were overcome and what elements need to be considered and their implementation. This presentation will also show how to engage those that have direct and indirect involvement in the animal care programme and communication strategies both internally and to the external environment. It will show how far we have come and the plans for further development in the future.

OD9S2

Culture of care in an AAALAC accredited, global contract research organisation

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Abstract
Culture of Care in animal research is recognized as an important component of organizational culture within the laboratory animal science and research community. This presentation will explore what constitutes an effective Culture of Care from the perspective of an AAALAC Accredited, Global Contract Research Organisation. Areas covered will include the value of an effective Culture of Care in terms of animal welfare, staff engagement, quality of research and compliance. This presentation will also share how a culture of care can be developed and nurtured in a global CRO, from the development of a vision that resonates well with people, how to take the pulse of a culture of care and establish a base line for future comparison, how to engage all relevant stakeholder to share ideas on approaches for supporting and embedding an widely embraced, vibrant and progressive culture of care. The role of the Establishment Licence holder, Animal Welfare and Ethical Review Body/Institutional Animal Care and Use Committee and Named People will also be explored including lessons learned to date.

OD9S3

Animal welfare standards and international collaborations

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Abstract
Globalization of the biomedical research enterprise is occurring at an accelerating pace. Increasingly, scientific collaborations and contracts cross national borders. Across the fields of science and engineering, an analysis of the number of publications authored by multiple international institutional contributors increased from 16.7% to 21.7% in the decade ranging from 2006 to 2016; in contrast, single-institution authorship declined. Signaling the importance of Asia to research collaborations is the rising number of publications from scientists in China, outstripping the U.S., and second only to the European Union. Assurance that the caliber of animal research and animal welfare is consistent among countries and that such animal use is done in a humane and conscientious manner is of significant concern to the scientific community, the general public and other stakeholders. However, the concept of ''animal welfare'' is often defined through the lens of cultural differences, shaped by economics, religious values and collective experience. It is an ethical imperative to design international collaborations in a manner that ensures high quality animal welfare. This outcome is critical in enhancing the validity of the data and reproducibility of results, thereby ensuring the animal use is both meaningful and impactful. This presentation will describe what key welfare elements need to be addressed when work is outsourced, how to establish consensus on good welfare, how to implement a higher standard of welfare in the collaborator's program, how to monitor welfare remotely, and steps that are being taken to harmonize animal welfare standards across the globe.

OD10S2

How we convinced ourselves of replacing sentinels by exhaust air particle polymerase chain reaction

Brielmeier Markus
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Abstract
Based on the drawbacks of sentinel monitoring in IVC reared rodent colonies we aimed at developing and testing better technologies. Sampling exhaust air particles at the prefilter of the IVC air handler with subsequent PCR of agent nucleic acids (EAP-PCR) turned out to be a promising method. EAP-PCR of highly prevalent agents (Mouse Norovirus, Pasteurellaceae, Helicobacter, Murine Astrovirus) under field conditions and in studies with known prevalence proved to be sensitive, fast and reproducible and was more reliable compared to sentinel monitoring. A comparison of sentinel and EAP-PCR monitoring in parallel over two monitoring periods in an animal facility consisting of 11 barriers showed clear superiority of EAP-PCR at lower cost. Sentinels, at the other hand, gave no relevant additional information. Consequently, sentinel use was stopped. EAP-PCR as the topmost monitoring level should be complemented by examination of sick or suspicious animals, where classical methods of serology, bacteriology and parasitology in addition to PCR are still state of the art. EAP-PCR methodology offers improved monitoring performance and contributes to the 3R through the reduction of sentinel mice.

OD10S3

Health monitoring of murine infectious agents via exhaust air particles

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Abstract
Exhaust air particle real-time polymerase chain reaction (EAP-PCR) is used to detect murine infectious agents. Two IVC rack vendors, A and B, developed in-line EAP collection devices with different air flow and mode of EAP capture. We compared their efficacies for detecting murine infectious agents using EAP-PCR. All materials used were decontaminated or sterilized. After rack decontamination, baseline samples were taken from each exhaust-air plenum and screened for infectious agents to verify the cleaning procedure. Over 3 months on each rack, singly kept naturally-infected male mice, primarily on a C57BL/6 background, and infected with *Helicobacter* (n = 24 or 21), *Staphylococcus aureus* (n = 9 or 5), *Pasteurella pneumotropica* (n = 25 or 22), *Streptococcus* beta-haemolytic (n = 30 or 23), *Klebsiella oxytoca* (n = 1) and their sentinel positive for *Entamoeba* spp. were kept in systems A and B, respectively. In both systems, 47/60 (A) or 47/70 (B) cages contained mice (n = 8 or 15 with negative mice). FELASA-listed infectious agents and *Bordetella bronchiseptica, Bordetella hinzii, Campylobacter genus, CAR bacillus, Corynebacterium bovis, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus* were monitored. The baseline samples from the racks tested negative. Both EAP capture media detected *Entamoeba* spp., *Helicobacter spp.* and *Pasteurella pneumotropica* but not *Staphylococcus aureus*, *Klebsiella oxytoca*, and group B beta-haemolytic *Streptococcus*. We showed, for the first time, that both EAP capture media comparably detected the infectious agents. The EAP-PCR technology serves as an adjunct method of health monitoring, leading to reduced numbers of mice used for routine health monitoring and contributes to the 3Rs.

OD10S4

Improving our health monitoring program by Exhaust Air Dust sampling

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Abstract
Housing rodents in individually ventilated cage systems (IVCs) presents some challenges for their effective microbiological monitoring, due to the system intrinsic characteristics of biocontainment and bioexclusion. The traditional approach, based upon the use of soiled bedding sentinels (SBS), is being progressively supplemented and, in some cases, also replaced by environmental microbiological monitoring. In particular, Exhaust Air Dust (EAD) sampling has been developed to increase the sensitivity of detection of a variety of microbiological agents and, at the same time, to reduce the number of animals used as sentinels.

The authors manage a 600 m^2 mouse facility housing around 6,000 ventilated cages used both for experimental activities and breeding of colonies of genetically modified mice. The traditional health monitoring program relied on SBS. In order to improve the effectiveness of the health monitoring program, a comparison between traditional SDS Vs PCR testing of EAD samples was carried out. The EAD sampling system tested allows to concentrate dust particles coming from all the IVC cages served by the air handling unit, on a dedicated sliding filter placed before the exhaust air pre-filter.

This new approach proved to be more sensitive than the traditional one, at least for the agents present in the mouse colonies housed by the facility. Based upon the positive results obtained, EAD sampling was introduced in the HM program of the facility partially replacing animal sentinels and allowing significant benefits not only in terms of application of the 3Rs but also in costs and labor savings.

OD10S5

Detection of lymphocytic choriomeningitis virus (and other agents) with filter media in IVC racks

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Abstract
Since nowadays, the prevalence of lymphocytic choriomeningitis virus (LCMV) in modern laboratory mouse vivaria is very low, it is difficult to obtain information whether this pathogen could be successfully detected with the EAD sentinel media, in contrast to other, more prevalent agents. A LCMV outbreak would have devastating consequences; therefore, it must be ensured that the health monitoring programme in use is capable of detecting it with reliable certainty.

Experimental infection of mice with LCMV in a biosafety level 2 (BSL2) environment is frequently used as a mouse model to study acute and chronic viral infections. Therefore, we used the opportunity to test the sentinel EAD capture system for its ability to detect LCMV infected mice in one of our BSL2 rooms.

Two racks were used, each containing 2 cages with infected mice, and one IVC rack with no infected mice. The other cages in the 3 IVC racks were used to host others study animals. The filter media were placed in each plenum of each rack. Six weeks later, three types of samples were collected: filter samples (n = 3), plenums swabs (n = 3) and feces (n = 6) from LCMV cages. These samples, placed in lysis buffer, were sent to the lab for real time PCR testing.

Results showed the detection of LCMV in 50% of each sample type (low copy numbers). The filter media are therefore suitable to detect LCMV after 6 weeks of dwelling time, even if only a small number of cages (2.5%) in the IVC rack is infected.

OD11S1

FELASA today and tomorrow – How can you contribute?

Santos Ana Isabel, Abelson K., Dorsch M., Gyger M., Mocho J., Pintado B. and Voipio H.
FELASA

Abstract
The Federation of European Laboratory Animal Science Associations – FELASA – is for the moment composed of 21 constituent associations representing 27 countries. FELASA was established in 1978 to represent common interests in the furtherance of all aspects of laboratory animal science (LAS) in Europe and beyond. LAS being that discipline whose objective is to ensure optimal conditions for the humane and appropriate use of animals for scientific purposes for the benefit of mankind and other animals, and to promote further development of those conditions. The FELASA board works:

- To advance and co-ordinate the development of all aspects of laboratory animal science and practice in Europe.
- To act as a focus for the exchange of information on laboratory animal science amongst European regions.
- To establish and maintain appropriate links with national, international and governmental bodies as well as other organisations concerned with laboratory animal science.
- To promote the recognition and consultation of FELASA as the specialist European body on laboratory animal science and welfare.
- To organise joint scientific meetings with constituent associations.
- To accredit courses according to the Directive 2010/63/EU.

Along the last 40 years there is a long list of achievements to share and a door open for new activities. FELASA working groups are established around current issues of LAS with experts in the field nominated by member associations. The working groups review available information and issue guidelines or recommendations promoting animals' health and well-being while keeping the highest standards of research.

OD11S2

Lessons learnt from the workshop on severity classification and reporting under Directive 2010/63/EU

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[1]*FELASA*
[2]*FELASA Severity Classification and Reporting Workshop core trainers*

Abstract
The workshop on severity classification and reporting under the directive 2010/63/EU is the result of the joint working group of FELASA, ECLAM, and ESLAV on ''Classification and reporting of severity experienced by animals used in scientific procedures''.

The working group report was published recently in a supplement of Laboratory Animals[1].

The working group members soon realized that classification of severity differs strongly between European countries. Therefore a standardized workshop was created by 3 highly experienced and dedicated core trainers in order to harmonize severity reporting across the European Union. Since then, many workshops have taken place all over Europe, mostly in conjunction with annual scientific meetings of FELASA member associations. More than 30 trainers have attended ''train-the-trainer'' workshops to further spread clones of the workshop by newly approved trainers in their home country.

During the workshop the legal and ethical basics for severity classification are communicated, followed by examples of procedures that are discussed and classified by the attendees. Prospective and actual classification of pain and suffering are performed.

To reach as many people as possible, examples have already translated into different languages. New examples are just being worked out.

The need for this workshop and its contribution to harmonize severity classification is unbroken.

The value and benefit of this workshop is also acknowledged by the European Commission in offering advertising on its website.

OD11S3

Joining forces to drive laboratory animal science harmonization

Mischler Scott
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Abstract
The Laboratory Animal Science (LAS) community represented by the Federation of European Laboratory Animal Science Associations (FELASA) in Europe and the American Association for Laboratory Animal Science (AALAS) in the United States have been working together to harmonize animal care and research recommendations. Joint activities began at the end of 2011 with the establishment of a Liaison Body comprised of leaders from each association. Several joint working groups have been initiated with the purpose of publishing recommendations pertinent to the LAS community. This session will provide a brief overview of AALAS, a synopsis of the structure and relationship between FELASA and AALAS, and a summary of this harmonization effort to the laboratory animal community.

OD11S4

The International Council for Laboratory Animal Science: Then, now, and looking forward

Pekow Cynthia
International Council for Laboratory Animal Science, Brussels, Belgium

Abstract
The role of the International Council for Laboratory Animal Science (ICLAS) has evolved over 50+ years. Originally called The International Committee on Laboratory Animals, the council was founded in 1956 by UNESCO, the Council of International Organizations in Medical Science (CIOMS), and the International Union of Biological Sciences. Part of the initial intent was to form an international body to survey and report back on the

production and use of research animals across the globe. Over time, the mission has changed to promote the development of laboratory animal science (LAS), particularly in areas with emerging economies, as well as to collaborate in harmonizing aspects of LAS that can improve research animal welfare and science. A strong example of an important collaboration can be seen in the ICLAS-CIOMS International Guiding Principles for Biomedical Research Involving Animals. ICLAS currently manages and assists with diverse programs, from assessing animal quality (rodent genetics and disease testing) to veterinary scholarships, to assisting with training and experiential opportunities in LAS across the globe. Challenges exist, particularly in such areas as funding and shifting political climate. Each new Governing Board works to collaborate with ICLAS members to promote initiatives that strengthen the ICLAS mission.

OD12S1

Muhlbock-Nomura Award: Experiences in lab animal diagnostics from 1982 to the present

Shek William R.
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Abstract
By the time I began my carrier in lab animal diagnostics in 1982, most mice and rats were being produced by the Cesarean-Originated Barrier-Sustained (COBS) process, developed by Dr. Henry Foster, the founder of Charles River, and other pioneers in the field of modern laboratory medicine that began after WWII. COBS rodents were referred to as specific pathogen-free (SPF) to indicate that they tested negative for infectious agents shown to interfere with research; that is, they tested negative for viral antibodies by serology, for pathogenic bacteria by cultural isolation (and phenotypic identification) and for parasites by gross and microscopic examinations. Assuming that most if not all indigenous rodent viruses had been discovered, Charles River referred to its barrier-reared, SPF rodents as VAF for viral-antibody-free. The replacement of conventional serologic tests by more sensitive solid-phase immunoassays (e.g., ELISA and IFA), however, provided evidence for the existence of novel parvoviruses found to be widespread in SPF colonies. The advent of molecular diagnostics in the 1990s, aided the discovery and characterization of the novel parvoviruses, followed by other prevalent and fastidious infectious agents, such as enterohepatic helicobacters, murine noroviruses (MNV) and astroviruses. The recent availability of affordable systems for massively parallel "next generation" sequencing has discovered many more indigenous rodent viruses, forcing us and others in biomedical research to reassess the practicality and importance of adhering to the dogma that SPF rodents should be free of exogenous viruses.

OD12S2

Bennet Cohen Award: Whole animal perspectives, ethical challenges and 3Rs enhancement using the five domains' model

Mellor David
Animal Welfare Science and Bioethics Centre, School of Veterinary Science, College of Sciences, Massey University, Palmerston North, New Zealand

Abstract
I have long adopted an integrated holist perspective with scientific topics and regard values (ethics) as providing essential support for animal-based science. In 1993 I developed the original Five Domains Model for assessing negative impacts of research, teaching and testing (RTT) procedures on sentient animals. At that time, most such systems focused on the precise target of the procedure, thereby neglecting additional negative impacts of the wider circumstances of the animals. The comprehensiveness of Five Domains Model addressed this deficiency. Also, impacts were evaluated in terms of the animals' likely experiences as opposed to what was done to them. This anticipated current acceptance that animal welfare refers mainly to the affective experiences sentient animals may have. From 1997, Model-assessment of all RTT procedures on sentient animals has been mandatory under New Zealand scientific procedures regulations. The Model targets Three Rs applications, especially Refinement, and supports thorough harm-benefit analyses conducted by institutional animal ethics committees. It has been regularly updated in line with the latest validated animal welfare science understanding. Thus, the list of negative experiences now considered has increased from the original six to 16, which improves targeting of Refinements. In contrast, validated affective neuroscience now supports behaviour-based environmental enrichments that increase opportunities for animals in laboratories to have positive experiences, giving impetus to such positive Refinement initiatives.Also, for 20 years the Model has increasingly been applied to welfare assessments well beyond the RTT sector; to farm, companion, sport/recreational, service, draught, wildlife (captive and free-living), 'pest', and other species.

OD13S1

Revised recommendations for health monitoring of non-human primate colonies (2019): FELASA Working Group update

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[4]Astrid Fagræus Laboratory, Comparative Medicine, Karolinska Institutet, Stockholm, Sweden
[5]Endell Veterinary Group, Salisbury, United Kingdom
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Abstract
The genetic and biological similarity between non-human primates and humans has ensured the continued use of primates in biomedical research where other species cannot be used. Health monitoring programmes for non-human primates provide an approach to monitor and control both endemic and incoming agents that may cause zoonotic and anthroponotic disease or interfere with research outcomes. In 1999 FELASA recommendations were published which aimed to provide a harmonised approach to health monitoring programmes for non-human primates. Scientific and technological progress, understanding of non-human primates and evolving microbiology has necessitated a review and replacement of the current recommendations.

These new recommendations are aimed at users and breeders of the commonly used non-human primates; *Macaca mulatta* (Rhesus macaque) and *Macaca fascicularis* (Cynomolgus macaque). In addition, other species including *Callithrix jacchus* (Common marmoset) *Saimiri sciureus* (squirrel monkey) and others are included. The important and challenging aspects of non-human primate health monitoring programmes are discussed. This oral presentation will summarize the broad lines of the FELASA recommendations which are simultaneously published in *Laboratory animals*. Main topics will include management protocols to maintain and improve health status, health screening strategies and procedures, health reporting and certification, and information on specific micro-organisms.

OD13S2

Non-human primates: Recommendations for breeding strategy – Use of F2 generation and higher for self-sustaining colonies

Decelle Thierry
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Abstract
As non-human primates provide essential models in research and as they are very sensitive species, high ethical and scientific standards are encouraged in the selection of laboratory primate suppliers. They need to comply with high standards of animal welfare, husbandry, veterinary management and health surveillance. As indicated in the European Directive – Article 28, breeding strategy should aim at increasing the proportion of animals that are the offspring of non-human primates that have been bred in captivity.

In its statement, FELASA supports the ultimate goal of the use of non-human primates that are the offspring of non-human primates which have been bred in captivity (generation F2 and higher) as set out in the Directive 2010/63/EU – Article 10 and its Annex II. To ensure a transition to this objective, FELASA has clarified the breeding strategy and the comprehension of self-sustaining colonies.

Reference
1. Statement on Self-Sustaining Colonies of non-human primates used for experimental or other scientific purposes, FELASA, 2018. http://www.felasa.eu/policy-documents/statement-on-non-human-primates/.

OD13S3

The road to success: Factors determining male introduction success in captive rhesus macaques

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Abstract
The entrance of new males into primate groups bears high social risks, yet migration is necessary to prevent inbreeding. Wild males may increase the likelihood of successful group entry by choosing a new group based on their own and the group's characteristics. In captivity, natural migration patterns are often mimicked to prevent inbreeding. Still, males are not always accepted in their new group. Applying knowledge of migratory choices from wild males to captivity may enhance male introduction success and long-term group stability. Therefore, we studied 64 male rhesus macaque (*Macaca mulatta*) introductions at the breeding colony of the Biomedical Primate Research Centre, The Netherlands. 49 (77%) introductions were successful, while the male obtained a long-term stable social position after 38 (59%) introductions. Introductions of experienced and heavier males, into groups with more adult females, but without pregnant females were most successful. Additionally, long-term group stability was highest when males were heavier, were at least 3.5 years old when they were removed from their natal group, and groups contained few matrilines and no pregnant females. This is in line with male migration patterns observed in the wild, and with the composition of wild macaque groups. These results highlight the importance of naturalistic group housing and mimicking natural migration patterns to increase male introduction success, and maintain long-term stable captive primate groups.

OD13S4

Monitoring social relationships in rhesus macaques

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Abstract
Fight injuries are a common problem in group-housed rhesus macaques and a significant welfare concern especially in breeding groups. Monitoring the social relationships in these groups is a key objective for improving our knowledge of why some groups fight more than others. We have developed methods that allow us to automatically identify and track rhesus macaques in video footage using face recognition. We will show an application of these methods to monitoring the social relationships in group-housed rhesus macaques.

Seven breeding groups of macaques were filmed for one week before and one week after their annual health screen (a perceived stressful event). Two cameras were used: the first camera focussed on the window area of the enclosure and the second on a high shelf. Both areas are commonly used by the monkeys for socialisation. The tracking software was used to identify which monkeys are in proximity to each other (with proximity used as a proxy for social behaviours such as grooming). We finish by discussing how these methods may be extended in future to detect specific behaviours including grooming.

OD13S5

Marmoset trends: Considerations and impact of introducing marmosets to a facility

Stathopoulos Jacquelyn
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Abstract
The common marmoset (*Callithrix jacchus*) is emerging as an invaluable model with numerous applications in neurocognitive as well as gene-editing research. As a result of their tremendous potential, use of these animals in biomedical research is currently experiencing a precipitous rise. Demand is currently outpacing supply, and is resulting in the establishment of marmoset colonies within conventional vivarium spaces, a decision that necessitates serious thought and consideration.

While their small size, social structure, and arboreal nature allow the utilization of a smaller cage footprint than other commonly used primate models, their modes of communication, which include high-pitched vocalizations, long distance alarm calls, and extensive scent-marking, may disrupt other animal species and research located in close proximity. Additionally, due to limited animal availability, many research programs elect to expand their marmoset census by establishing breeding colonies – a time and labor-intensive endeavor.

The successful integration of a marmoset colony into an existing vivarium space requires preemptive considerations of room and cage densities, the appropriate management of noise and odors, the creation of room level infrastructure that reliably maintains an optimal housing environment, the design and construction of dedicated clinical treatment and surgical spaces and procurement of associated equipment, and the training of technicians in both routine marmoset care and specialized tasks such as infant rearing. Each of these topics will be fully discussed to allow informed strategy and effective implementation.

OD14S1

If not CO2, then what: Does any alternatives qualify as a refinement?

Weary Daniel
Animal Welfare Program, University of British Columbia, Vancouver, Canada

Abstract
The first presentation in this session reviews the scientific evidence regarding the humaneness of CO2 as a euthanasia agent. This presentation is intended for audience members that conclude, on the basis of this evidence, that there are significant welfare concerns associated with the continued use of CO2 for this procedure. I will review the scientific evidence regarding alternatives, and for each I will critically examine if the method can be considered a refinement over the use of CO2 alone. To do so I will discuss the strength and weakness of different scientific approaches used to assess euthanasia methods, what qualifies as 'refinement', and what criteria can be used to decide if the magnitude of refinement is sufficient to merit a change in procedure. I conclude that, of the inhalation methods available, the use of halogenated anesthetics meets the standard of a refinement over the use of CO2 alone for killing rodents that are naïve to both agents. I also briefly discuss the use of physical methods and discuss the need for new research in this area.

OD14S2

Welfare impacts of carbon dioxide for killing laboratory rodents: Evidence for CO2-induced anxiety/panic

Golledge Huw
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Abstract
Most laboratory rodents are killed with Carbon Dioxide (CO_2). It is well-established that CO_2 causes pain in high concentrations (>∼50%). Pain can be avoided by using a rising concentration of CO_2, inducing anaesthesia at ∼40%, before CO_2 reaches noxious levels. Low CO_2 concentrations (∼5-15%) cause other welfare impacts including aversion, probably due to fear/anxiety. These concentrations are below those which induce unconsciousness, meaning all rodents killed with CO_2 probably experience poor welfare. Compelling evidence CO_2 causes aversion comes from experiments which characterise its effects as a killing agent, showing that rodents forego rewards or expose themselves to stressful environments to avoid CO_2.

Less well-appreciated is extensive evidence from pre-clinical pharmacology studies that CO_2 is an anxiety- or panic-inducing agent. CO_2 is used to model anxiety/panic disorders. Humans exposed to low concentration CO_2 experience fear or anxiety. Similarly, rodents exhibit fear or panic-like responses (conditioned and spontaneous freezing, avoidance of open spaces) upon exposure to low concentration CO_2. Such responses are ameliorated by anxiolytics. Brain regions involved in affective responses mediate behavioural responses to CO_2 in rodents, and specific ion-channels

responsible for detection of CO_2-induced acidosis which when knocked-out reduce CO_2-induced fear responses have also been identified.

This evidence, which I will summarise, suggests that although minor refinements (e.g selection of optimal CO_2 flow rate) can (and should) be made, there is no way to refine the use of CO_2 to the point where it can be considered a truly humane killing agent.

OD15S1

Recommendations for the rehoming of research animals: Implementation of a Working Group

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[6]*Laboratory for Ethology,, Faculty of Veterinary Medicine, Ghent, Belgium*

Abstract
Article 29 of the European Directive promotes rehoming and socialisation of research animals as a new responsibility for laboratories and Article 27 enhanced the role of Animal-Welfare body to advise on these topics.

Although rehoming can be appealing, with potential benefits for both the animals and the institution, in practice, even in facilities where the process has been established for many years, successful rehoming can still be a challenge. Currently, depending on national regulations and the species involved, each institute has to manage rehoming within its own constraints.

Faced with these findings, a Working Group has been appointed by FELASA to draw up recommendations for guidance in implementing rehoming.

While considering the unharmonized national regulations in this field, the Working Group will define criteria for rehoming, taking into account the species concerned and develop recommendations on management of animals throughout their life in the facility, to offer them the best chance of inclusion in a rehoming program. For some species as rodents, fishes or farm animals, the opportunity to include them in a rehoming program has to be evaluated.

The criteria for selection of potential adopters and the best method to raise their awareness of potential problems that might occur with rehomed animals will also be addressed in these recommendations.

OD15S2

Rehoming of former laboratory primates: Dutch code of practice

Louwerse Annet
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Abstract
The aim of ''rehoming'' is to guarantee the quality of life of animals that remain alive at the end of an experimental procedure. When rehomed, an animal should be able to spend the rest of its life at a location suitable for its needs, without being subjected to any further animal procedures. However, for various reasons, not all animals that remain alive at the end of a procedure are – or can be – rehomed. In the case of Non Human Primates (NHP's), the process is complicated because the scope of the provisions differ from those for domesticated and companion animals. In the Netherlands, NHP's are not offered to individuals and must always be cared for and supervised by professionals.

Rehoming NHP's should only be done after careful consideration of all current and future conditions and circumstances concerning the individual animal. In the Netherlands, the main factors to be considered for rehoming NHP's are set out in a framework. The competence of the licensee to release animals; the selection of suitable animals; the preparation for rehoming; the assessment of the suitability of the new living environment; the follow-up and the financial and legal aspects are well described in the Dutch Code of Practice. The Dutch code of practice can set an example for rehoming NHP's worldwide.

OD15S3

Rehoming protocol for research dogs and cats at Ghent University in Belgium

Moons Christel P. H.
Nutrition, Genetics and Ethology, Ghent University, Merelbeke, Belgium

Abstract
The research animal rehoming programme of the Faculty of Veterinary Medicine at Ghent University consists of: screening research animals when they become available for adoption, matching of potential adopters with a particular animal in terms of the home environment and adopter expectations, educating potential adopters, and signing of a rehoming contract.

Because dogs and cats live in close contact with humans (adults and children) and possibly other animals, the difference between the research environment and the environment post-rehoming is significant. An additional step in the adoption programme for these species, therefore, involves observation of the animal in different contexts to assess the behavioural strategies an animal is likely to adopt post rehoming, and a meeting between potential adopters and an animal behaviour expert. During this meeting, particular attention is paid to educating potential adopters about canine or feline stress signalling, and to motivate them to use this knowledge to facilitate the adjustment process.

In case the dogs or cats have a health issue that requires care and follow-up by the adopter, the first step in the rehoming process is to assess the suitability for adoption by a working group within the Ethical Review Body.

OD15S4

Implementation of a three-part rehoming programme for dogs

Roth Mirjam
animal aspects, Biberach an der Riss, Germany

Abstract
Appropriate socialisation for dogs prior to rehoming is required by article 29 of the European Directive. To ensure this requirement is met, socialisation of dogs should be integrated early in the animal's laboratory life. The limited variety of stimuli in research settings compared to a domestic environment must be specifically considered.

In the presentation a three-part programme will be introduced consisting of **basic behavioural training** and **domestic environment desensitisation** (habituation). Both begin when the dog arrives at the facility. The third section includes a **transfer preparation phase** that takes place prior to the actual rehoming. This will not be addressed any further in the talk.

The first part (basic training) includes behaviours that support everyday handling and husbandry such as good dog manners (greeting with 4 paws on the floor, say please by sitting) and elements of basic obedience like loose leash walking. These will be discussed in the talk using examples. Adopters can continue this at the time of rehoming, significantly supporting the acclimatization process. This aspect of a rehoming programme has benefits throughout the dog's laboratory life because it facilitates daily animal care, prevents stress through predictability and control, provides cognitive enrichment and due to the training history, will speed up study related trainings.

The second part (environmental desensitization) aims to provide a spectrum of environmental variance into the laboratory routine by integrating auditory, visual, olfactory and tactile stimuli (sound socializing CD's, paw path etc.) and will be presented with practical examples.

OD15S5

Investigating the outcome of a laboratory dog rehoming programme in Ireland

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[2]*Research and Veterinary Services, University of Edinburgh, Edinburgh, United Kingdom*
[3]*Bright Dogs, Scotland, United Kingdom*

Abstract
Laboratory animals are usually euthanised at the end of a study, yet rehoming can be an alternative option. There is a paucity of literature and guidance on rehoming and little data regarding the numbers of animals being rehomed internationally, suggesting it is uncommon. This study examined the outcome of a laboratory dog rehoming programme, where 346 dogs were transferred from a research laboratory into animal shelters for private adoption. Permission was granted to send 88 adopters a questionnaire asking them to report on motivations for adopting, their dogs' behavioural characteristics, and their expectations and satisfaction. The response rate was 67.9%. Separately, out of a sample of 207 other adopters, data were collected regarding returns to the shelter.

Adopters were highly satisfied, rating satisfaction with their dog 9/10 on average. Aggression was uncommon and most dogs were friendly. The most common undesirable behaviour was fearfulness, which appears more prevalent in laboratory compared to non-laboratory dogs. One third of owners had underestimated the effort involved in caring for their dog, and these owners were significantly less satisfied.

The return rate of the 207 dogs was 10.6%, with just over half of these for behavioural reasons, consistent with the literature for general dog adoptions.

Other than the high prevalence of fearfulness, which should be explored further, the findings are similar to those of other dog adoption studies, indicating that laboratory dogs can make good pets. This study recommends that adopters are provided with more information and support, in order to better manage expectations.

OE1S1

Norecopa: Experiences with building 3R databases and international networks

Smith Adrian
Norecopa, Oslo, Norway

Abstract
Norecopa has maintained a database of 3R resources since 1991, at which time the presentation of data on the Internet was still in its infancy. Now, with the emergence of powerful, free search engines and the presence of seemingly unlimited data on the internet, 3R databases and international networks need to demonstrate «real added value», with a focus on best practice and quality, if they are to be attractive. An international network of laboratory animal specialists is crucial role to the success of this work – both to secure the financial footing and for the identification of quality material for the database.

Recently, we have witnessed the establishment of an international Culture of Care Network, the emergence of many new European 3R centres and great interest in collaboration between these centres. In our experience over the last 25 years, the initial enthusiasm for such projects must be followed up by the development of a sound financial footing and a very specific and relevant agenda, if they are to remain active over time.

Email discussion fora such as CompMed, LAREF and VOLE have played important roles in the dissemination of knowledge which is needed for projects like Norecopa's database. Norecopa would like to see a similar forum specifically for laboratory animal staff in European countries. This might aid the work of harmonisation of

the procedures needed to implement EU Directive 2010/63 in all Member States.

OE1S2

Centro 3R: Progress and hurdles in implementing 3Rs teaching and research across Italian Universities

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[1]*University of Pisa, Pisa, Italy*
[2]*University of Genova, Genova, Italy*
[3]*Polytechnic of Turin, Torino, Italy*
[4]*Polytechnic of Milan, Milan, Italy*

Abstract
The Italian Interuniversity Center for the promotion of 3Rs principles in teaching and research (Centro 3R) was established in early 2018. It currently boasts 4 member universities (university of Pisa, University of Genova, Polytechnic of Milan, Polytechnic of Turin), over 250 members and brings together a wide range of expertise in disciplines such as pharmacology, engineering, law, biology, medicine and philosophy.

A characteristic of the Centro 3R is its inclusivity and its scientific, rational and evidence-based approach to the question of humane experimentation in all fields of research. In fact, acknowledging that animal research is still an important way of building knowledge on unanswered biological questions, it seeks to sensitize researchers to humane methods and humane ways of approaching science. Thanks to this inclusive and open approach, it is becoming a point of reference for teaching resources in Italian academia and a platform for discussions.

Among its activities are an annual course on in vitro methods and the creation of a pool of elective courses on topics related to the 3Rs. A description of the Center, its structure and networks as well as its events will provided with a discussion of the state of the 3Rs in the Italian landscape and the hurdles we encounter in promoting 3Rs.

OE1S3

The bridge inbetween – 3R Centre Giessen – interconnecting 3R research

Jedlicka P.[1,2], Zintzsch A.[1] and **Krämer Stephanie**[1,3]
[1]*ICAR3R – Interdisciplinary Center for 3Rs in Animal Research, Justus Liebig University Gießen, Giessen, Germany*
[2]*Professorship for Computer-Based Modelling in the field of 3R Animal Protection, Faculty of Medicine, Justus Liebig University Gießen, Giessen, Germany*
[3]*Professorship for Laboratory Animal Science and Animal Welfare, Veterinary Medicine, Justus Liebig University Gießen, Giessen, Germany*

Abstract
Despite the prevailing consensus, both in the scientific landscape and in social and political perception that implementation of the 3R concept is of uttermost importance, there are still obstacles to sustainable implementation, especially in biomedical research. The aim of the newly founded, interdisciplinary 3R Centre Justus-Liebig University Giessen is to develop innovative didactic methods actively implementing the 3R ideas of Russell and Burch. Thereby, the focus lies on practical application and development of suitable alternative methods and the implementation of refining procedures to visibly improve animal welfare. The strength of the centre is its high degree of interdisciplinarity. This is not only displayed by the fact, that the centre is a collaboration between two university departments (veterinary medicine/ medicine), but also by the support of 3R research by other departments like the faculty of experimental physics and material testing. Furthermore, there is a close association with the department of philosophy and humanities to reflect ethical issues of human animal relationships in the critical context of animal testing.

To make this possible, the State of Hesse supported the implementation of three new 3R professors, focusing on the aspects of refinement, replacement (alternative drug testing) and reduction (in silico methods). In addition to the didactic orientation, the centre pursues active 3R research. Thus, the unique interdisciplinary orientation and university-associated connection of the centre guarantees an innovative realization and further development of the 3R concept.

OE1S4

The Danish 3R-Center – Initiatives and results

Bollen Peter and Kornerup Hansen A.
The Danish 3R-Center, Copenhagen, Denmark

Abstract
The 3R-Center started in 2013 to promote the 3Rs by initiating research projects focusing on one or more of the 3Rs and by disseminating knowledge and best practices of the 3Rs in Denmark.
Organization
The 3R-Center is established by the Ministry of Environment and Food. The board consists of seven impartial, scientific members. The center has a secretariat and a budget of its own, but is also supported by industry and animal welfare groups.
3R-symposium
The annual international symposium is a significant event where researchers, veterinarians, animal caretakers, and other people with an interest in 3R meet and network. The program is composed of Danish and international speakers and covers all three Rs. In 2019 the symposium will take place on November 12–13.
3R-survey
The Center has funded two studies which 1) examined the Danish stakeholders' knowledge of and experience with the 3Rs and 2) examined factors which either inhibit or promote the implementation of replacement in research.
Research funding
Each year the 3R-Center funds 3R related research projects. Funded projects must be of a high scientific quality and relevance. Every year 1,5 million DKK is reserved for research funding.
Teaching material

The 3R-Center has prepared teaching material for use in Danish lower and upper secondary classes. The material is available online and free of charge. The material is also available in a printed version.

3R-News

The 3R-Center's website focuses on disseminating research and news relevant to persons interested in or working with 3R and laboratory animal science www.en.3rcenter.dk/

OE1S5

Outstanding research and education in Berlin leads to the "Capital of 3R"

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[2]*Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany*

Abstract

Outstanding research in Berlin with seven Clusters of Excellence approved by the German Federal and State Government requires a center for the development of animal-friendly research according to the 3R principle.

Established in 2014 by funding of the Federal Ministry for Education and Research, the Berlin-Brandenburg research platform BB3R integrated the research activities of the Freie Universität Berlin, Charité – Universitätsmedizin Berlin, Federal Institute of Risk Assessment, Technische Universität Berlin, Zuse Institute Berlin, and Universität Potsdam. From the beginning, BB3R trained the next generation of scientists by introducing the first graduate school worldwide on 3R in science and education. The established graduate program qualifies students in modern techniques in biomedical sciences, pharmacology, and toxicology for a subsequent career in the field of 3R-related life sciences or scientific administration.

Starting in 2018, Charité3^R aims at bundling and coordinating interdisciplinary research at Charité – Universitätsmedizin Berlin with emphasis on the 3R and translation improvement in biomedicine: finding the best therapies by using animal-free methods whenever possible, establishing meaningful human disease models, and increasing animal welfare. As an interdisciplinary and interdepartmental organizational structure, Charité3^R will support researchers in 3R projects, implement the 3Rs in education together with BB3R, and communicate 3R research challenges and needs to the public.

BB3R and Charité3^R will hence be the nucleus of a 3R network, which will promote Berlin in becoming the ''Capital of 3R'' with outstanding research and collaborations with local, national, and international partners from public research institutions and pharmaceutical or biomedical companies.

OE1S6

The Austrian 3R Centre – The progress of linking scientists and the 3Rs

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[3]*Division of Hygiene and Medical Microbiology, Medical University Innsbruck, Innsbruck, Austria*

Abstract

The Austrian Centre of the 3Rs is an umbrella organisation that has emerged from a local initiative of the three Austrian medical universities. The RepRefRed Society and the animalFree research Cluster of the Medical University of Innsbruck were combined in this umbrella organization to work on and promote the 3Rs principles, founded in 1954 by William Russel and Rex Burch.

The Organisation focuses on active exchange of information among researchers through topical meetings and events. The resulting increased transparency of protocols is intended to bring not only "refinement" via improvement of animal welfare but also "reduction" in the number of animals used in experiments. The MUI animalFree Research Cluster focuses on replacement and establishes alternatives to animal testing not only in pharmaceutical but also in basic and translational context via knowledge exchange between researchers and in teaching medical students. First goals of the Austrian Centre were to establish a uniform training in animal experimentation, which equals the working document on education in animal experiments, as well as implicating uniform trainings with respect to alternatives to animal testing.

Animal welfare committees of all Austrian Universities were invited 2017 to work on training guidelines. At the end of 2019, these guidelines are to be adopted and implemented by all universities. 3R days were established in 2017 as well as Austrian wide meetings with respect to alternatives to animal testing as a communication platform between researchers, veterinarians and animal welfare committees. Meanwhile these meetings have grown from local interdisciplinary meetings into Austria-wide events.

OE2S1

Fundamental and diverse: Insights to biological rhythms from studying wild species

Helm Barbara

Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, Groningen, Netherlands

Abstract

Life on earth is inherently rhythmic. In adaptation to the geophysical cycles of their environments, organisms undergo periodic changes that range from social behaviour down to gene expression. Biological rhythms are organized by complex time-keeping

mechanisms, which combine endogenous rhythms with selective responsiveness to environmental factors. Responses to the environment can be exquisitely sensitive, and they typically fluctuate depending on the phase of the rhythm within an individual. Moreover, although biological rhythms are deeply shared between organisms, natural selection has greatly diversified the cycles and sensitivities depending on a species' lifestyle. Per implication, environmental change, experimental treatments and captive holding conditions can all have substantial and unforeseen consequences for animals and experimental outcomes, unless the underlying rhythmicity is well understood. Broader insights on rhythms in different species and environments therefore have the potential to improve life of humans and laboratory species, as well as help to mitigate effects of anthropogenic change in the wild. I will give an overview of the diversity in patterns and mechanisms of biological rhythms, and then focus on avian research carried out on captive and wild species. I will focus on daily (circadian) and annual (circannual) rhythms of birds and on their response to altered environments. The overall intention is to inspire ideas for mitigating conflicts between ancient time-keeping systems and anthropogenic living conditions.

0E2S2

Research and management of wild animals: Politics, ethics and the 3Rs

Smith Adrian[1] and Arnemo J.M.[2]
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[2]*Faculty of Applied Ecology, Agricultural Sciences and Biotechnology, Inland Norway University of Applied Sciences, Elverum, Norway*

Abstract
Conflicts between human settlements and wildlife populations are on the increase, fuelled by expansion of residental areas and less tolerance for those species which are perceived, rightly or wrongly, to be a threat to human health. These conflicts often raise issues which politicians wish to tackle quickly, while scientists will often prefer to evaluate the situation over time before taking a standpoint. Differences between the way in which neighbouring countries tackle the authorisation of wildlife capture illustrate the dilemmas inherent in this work.

In addition to the political and scientific arguments for and against a common set of rules for project authorisation, comes the scientific evaluation of the procedures used for capture, immobilisation, anaesthesia and analgesia in the field. Many of these methods have been based upon tradition, with an acceptance of standards which would be considered totally unacceptable in modern clinical veterinary practice or in human medicine and surgery. There has been, and still is, a great need for detailed, species-specific guidance in this area and closer cooperation between scientists and suppliers of technical solutions. For example, the available guidance on severity classification, now mandatory under Directive 2010/63, is often perceived as having little relevance to field studies. Consensus meetings on the care and use of animals in field studies have been arranged by Norecopa to aid the work of implementing the 3Rs and solving the issue of varying standards for project evaluation and authorisation. This presentation will present some of the conflicts and possible solutions within this area.

0E2S3

Wild at heart: Improving fish welfare in regulated procedures in the wild

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[2]*Joint Production System Ltd, Potters Bar, United Kingdom*

Abstract
Electronic telemetry has provided an unprecedented understanding of the movements, behaviour, life history and survival of fish, free-ranging in their natural habitat, information that is directly relevant to their management and conservation. At sea, commercial fishing vessels are often used to capture the study species of fish. On deck, fish undergo a regulated procedure under the Animals (Scientific Procedures) Act 1986, to attach electronic Data Storage Tags (DSTs), before releasing the fish back to the wild. As tagging methodologies and technologies evolve, it is important to review best practise to minimise negative effects on the fish's behaviour and survival, the very parameters being measured, as well as its long-term welfare.

It is challenging to perform regulated procedures outside of a laboratory environment, in the wild, ensuring the best possible handling and welfare standards. It's not possible to achieve entirely benign capture methods or truly aseptic conditions for surgery at sea, but with planning and fore-thought, high standards of surgery and welfare can be achieved. Our experience with tagging marine finfish provides some guiding principles of refinements in handling, anaesthesia, analgesia, asepsis, and species-specific vitality, reflex and injury assessments to assess fish fit for release to the wild.

Our refinements improve methods that provide a fundamental understanding of the movements, distribution and survival of fish in relation to their environment. They benefit not only the welfare of the tagged fish, but also the advice that feeds into UK and European policy, influencing the assessment, management and conservation of marine fish stocks.

0E3S1

Animal welfare bodies: Challenges and strengths – A round table discussion

Jerchow B.[1] and Olsson Anna[2]
[1]*University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany*
[2]*IBMC – Instituto de Biologia Molecular e Celular, Porto, Portugal*

Abstract
The function of the animal welfare body (AWB) is to work towards a maximum of animal welfare within an establishment. Optimally, it should be the starting point and catalyst of a Culture of Care. To achieve its goals, the AWB is supposed to provide advice on the

welfare and care of the animals, the application of the 3Rs, review management and operational processes, and follow the development and outcome of projects. In practice, AWBs are made up of a number of stakeholders with different agendas, some of which may potentially conflict with the promotion of a Culture of Care. In form of a round table we want to discuss amongst the panel and the audience, how AWBs can become a resource rather than an obstacle, how interaction with other entities – other AWBs, the National Committee, etc. – can be fostered, and how the work load caused by the commitment to an effective AWB can be handled. The panel brings together the experience and perspectives of an animal technician, a biomedical researcher, an animal welfare scientist, a national committee member and an animal welfare officer from different European countries.

OE3S2

The Beyond animal testing index: How to assess your institutes contribution to the 3Rs?

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[1]*Leiden University Medical Centre, Leiden, Netherlands*
[2]*University of Applied Sciences, Utrecht, Netherlands*
[3]*The Dutch Society of the Replacement of Animal Testing, Den Haag, Netherlands*

Abstract
The transition to animal free innovation is an ambition with some urgency in The Netherlands and at the level of the European Union. Animal free innovation is not a new initiative. They are generated where science and technology meet. Sometimes as a result of purposeful researching and building, more often in a more serendipitous way. The Beyond Animal Testing Index (BATI) has been developed as a benchmark for research institutes involved in animal experimentation to compare their respective contributions to developing and implementing animal free innovations and in a more general sense to the 3Rs based on the information already present in academic institutes in the Netherlands. The BATI is developed after the Access to Medicine Index, which benchmarks pharmaceutical companies according to their efforts to make their medicines widely available also in third world countries. The BATI was designed and drafted in 2018. The draft BATI was presented to stakeholders. Their feedback was included to produce the first version of the BATI. This version will be presented including an assessment of its feasibility. As a benchmark tool, the BATI is expected to act as a stimulus for research institutes to learn and improve on their strategies towards the transition to animal free innovations and the 3Rs.

OE4S1

Review of Directive 2010/63/EU

Louhimies Susanna and Anderson D.B.
Directorate-General for the Environment, European Commission, Brussels, Belgium

Abstract
Directive 2010/63/EU took effect in January 2013. Article 58 required that the Commission review the Directive by 10 November 2017. Information was sought from Member State authorities, stakeholder organisations and from establishments authorised to use, breed and/or supply animals for use in scientific procedures across the EU. On the basis of the draft findings, an open meeting was convened by the EC in March 2017, to provide an opportunity for stakeholders to comment on these findings. Many considered the timing of the review premature as experience of the new provisions was still limited. Furthermore, the level of implementation varied vastly depending on both the previous legislation in place and the speed of progress made with the transposition of the new requirements into national law. Nevertheless, there are several provisions already developing and working well, such as the introduction of Animal Welfare Bodies, which are contributing positively to animal use and care practices. Others include increased awareness of the Three Rs and growing recognition within research community of the important link between good science and animal welfare. However, the review identified a number of areas where further progress is required if the key objectives of the Directive are to be met. For example, the requirements for project evaluation and authorisation, and education and training vary considerably. A number of recommendations were made, addressed to Member State authorities, regulators, researchers and stakeholder organisations. The presentation will provide an update on progress with the uptake of these recommendations.

OE4S2

Pharmaceutical Industry efforts to work on Directive 2010/63/EU Review Recommendations

Reid Kirsty
Science Policy and Regulatory affairs, EFPIA, BRUSSELS, Belgium

Abstract
At EFPIA (European Federation of Pharmaceutical Industries and Associations) there is a foreseen importance of promoting good science and animal welfare, as well as increasing understanding of how the two are intertwined.

Medical research continues apace. Although pharmaceutical companies cannot avoid the use of laboratory animals to prove that medicines work, it is our priority to meet high standards of animal welfare. To put animal welfare principles into action, we systematically implement alternative methods where possible and improve standards of care.

The Directive 2010/63/EU sets full replacement as a long-term vision and has three ambitious goals on harmonization, application of 3Rs and transparency.

There is already evidence of positive impact however a recent review of the Directive indicated there is potential for further improvement whereby the Commission listed recommendation which require take-up and collaboration between all stakeholders and disciplines. A significant number of these recommendations are addressed to scientific community itself. Furthermore, the implementation of some of the proposed recommendations requires support from Commission, competent authorities, national animal welfare committees, animal welfare organisations, 3Rs centres, etc.

The pharma sector is undertaking numerous activities and actions on these recommendations and these will be addressed during the presentation.

OE4S3

Directive 2010/63/EU as a driver towards the ''full replacement of procedures on live animals''

Bastos Luisa F.
Eurogroup for Animals, Brussels, Belgium

Abstract
The EU Directive for the protection of animals used for scientific purposes created mechanisms that, well used, can aid the scientific community to achieve one of its goals: Guarantee that animals are used as the very last resort. These mechanisms can be powerful tools to help define strategies to move away from the use of animals in specific areas of research, but improvements are needed.

Challenging the need for the use of animals in specific projects or research areas is an important role of animal welfare bodies, project evaluators, national committees, and competent authorities. However, the first review this Directive pointed out concerns with the efficiency and consistency of project evaluation and authorisation processes. The outputs of project evaluations are maybe being affected by different contexts as proximity and expertise of the evaluators, and adopted processes and guidelines.

Another concern raised by the Commission's review was that the adoption of alternative approaches was not being ensured. Independent and consensual thematic reviews (article 58) can provide the opportunity to scrutinise concrete scientific areas where animals are being used, and analyse the potential to move towards non-animal approaches.

This and other mechanisms to carry out periodic reviews can lead to scientific policies that promote innovation ''towards achieving the final goal of full replacement of procedures on live animals'' (in recital (10)). In this presentation we will look into the progress that has been achieved in the last years and explore how different review mechanisms can further improve the implementation of this Directive.

OE5S1

The Harm-Benefit Analysis: Towards an adequate and standardized methodology

Grimm Herwig
Messerli Research Institute, Veterinary University Vienna, Medical University Vienna, University Vienna, Vienna, Austria

Abstract
Anyone aiming at a thorough understanding of the Harm-Benefit Analysis (Art. 38 (d) Directive 2010/63/EU) has to deal with at least the following four components (Grimm/Olsson/Sandøe 2018): a) a defined set of criteria which comprise the harm and benefit dimensions to be included; b) the relative weights of the individual criteria; c) operational factors to identify and measure how well each criterion is full-filled; d) a procedure that aggregates total harms and benefits into a final HBA outcome. Implicitly or explicitly these factors play a significant role in metric and discourse models of the HBA. However, this is by no means a trivial enterprise and it is highly doubtable that all the member states' national authorities and their supporting bodies follow similar standards when carrying out the HBA. Therefore, the question emerges whether applications with comparable harm and benefit are treated unequally among member states.

Against the background of an Austrian project to develop a metric methodology to systematically carry out the HBA, this contribution aims at identifying some major pitfalls but as well steps towards an adequate and standardized method to carry out the HBA in order to harmonize the project evaluation across member states and the HBA in particular. First, metric and discourse models and their pros and cons will be debated. Second, an adequate understanding of benefit assessment will be argued for. Building on these pillars, a more promising methodology for the HBA will be outlined and discussed.

OE5S2

Promoting consistency in project evaluation

Ryder Kathryn
Home Office, Dundee, United Kingdom

Abstract
As a regulator with currently 21 inspectors performing project evaluation (PE) sometimes in remote locations across UK, the Home Office promotes consistency by:

- Recruitment of PEs ensuring that there is broad experience in relevant fields
- formalised inspector training in PE on induction with learning outcomes and practice exercises
- oversight by a senior inspector, ensuring supervision until competent
- a standard form must be completed which confirms / verifies / evaluates all legal requirements and the harms and benefits
- annual appraisal includes a level of oversight into quality of project evaluations
- Continuous Professional Development Meetings 4 times a year when topics relevant to project evaluation are discussed
- collaboration with other evaluators when novel or contentious projects require evaluation (second opinions)
- National Committee referrals for contentious projects or those with potential societal concern providing a level of independent oversight of the outcomes of project evaluation

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OE5S3

Diversity in project evaluation

Olsson I., Anna S.[1], Silva S. S.[2], Lassen J.[3] and Sandoee P.[3]
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[2]*ISPUP, Universidade do Porto, Porto, Portugal*
[3]*University of Copenhagen, Copenhagen, Denmark*

Abstract
The fundamentally revised and expanded European legislation (Directive 2010/63//EU) protecting animals used in research now covers evaluation and authorization procedures, and includes aspects that were not included in the previous Directive (86/609/EU). These aspects (e. g. predicted benefit, 3Rs compliance, severity, harm-benefit analysis) correspond to what is typically covered by an 'ethics review', although this term is not used in the Directive. How and by whom projects are to be evaluated was left to individual Member States (MS) to determine, which has resulted in considerable diversity in the approach to evaluation and authorization. The two most common approaches are i) that the projects are only evaluated and authorized at a national level (the evaluation is conducted by a national committee and the authorization provided by the national competent authority) and ii) that the projects are evaluated at institutional, local or regional level (by a committee) combined with an evaluation at a national level (by a committee and/or officers) with the authorization being provided also at a national level.

Committee size and composition also vary. Most committees include scientific expertise and veterinary expertise, whereas expertise in law, ethics and alternatives to animal experiments is less common. Committee members from outside the scientific and technical community are most often representatives of special interest groups (mainly animal welfare associations), whereas genuine lay members are rare. In the presentation, we will discuss potential underlying reasons for MS opting for these different approaches, and the consequences for the project evaluation process.

OE5S4

Why consistency in project evaluation is important

Anderson David and Louhimies S.
European Commission, DG Environment, Brussels, Belgium

Abstract
Directive 2010/63/EU requires that all projects are subjected to a project evaluation and sets out in detail the elements to be considered, and the type of expertise which should be considered when conducting such an evaluation. The evaluation requires that a harm – benefit analysis forms part of the process, to assess whether the harm to the animals is justified by the expected outcomes, taking account of ethical considerations. Project Evaluation is a cornerstone of the Directive in ensuring the Three Rs are implemented and animal welfare compromises minimised consistent with the scientific objectives. Consistency in the outcomes of this process is important in ensuring delivery of a level playing field for the scientific community, as well as confidence in the regulatory framework. Several factors influence consistency, starting from the definition of the term ''project''. In addition, the level of detail in which projects are scrutinised plays an important role. An equilibrium needs to be established that minimises administrative burden whilst obtaining assurance of a robust scientific design, implementation of the Three Rs and animal welfare obligations in line with the Directive. The presentation will explore these issues and other difficulties raised by the scientific community in the Directive review and how these may be addressed.

OE6S1

Focus on transparency – Non-technical project summaries under Directive 2010/63/EU

Louhimies Susanna and Anderson D. B.
Directorate-General for the Environment, European Commission, Brussels, Belgium

Abstract
Transparency is essential to develop a trust in the systems of ethical and socially acceptable care and use of animals in science as the basis for the continued research using animals in the EU until such time that their use can be replaced by non-animal alternatives. Comprehensive and accurate information is a prerequisite for decision making, be it for policy development, research funding or simply to understand the status quo. Accordingly, improved transparency was set as one of the key objectives for Directive 2010/63/EU. The tools to facilitate improved transparency include the revised requirements for statistical reporting, and the publication of non-technical project summaries to provide objective information on projects using live animals. The review of the Directive, completed in 2017, showed that majority of Member States and users considered that the publication of non-technical project summaries was already positively contributing to transparency, although the full impact had yet to be realised. However, not all stakeholder groups, especially those representing animal welfare, shared this view. The main issues raised concern the accessibility, both in speed and ease of access, the quality of content and absence of any facility to search them at EU level. One of the recommendations of the review called for the Commission, Member States and stakeholders to explore the possibilities of a central EU repository of non-technical project summaries. The talk will present the actions the Commission has taken in this regard and the progress towards improving both the accessibility and quality of non-technical project summaries.

OE6S2

How to consider non-technical summaries: Tolerance toll, translational task or transparency tool?

Gyertyán István
National Scientific Ethical Committee on Animal Experimentation, Budapest, Hungary

Abstract
Although the requirement of non-technical project summary (NTS) may seem to be a simple matter, as the competent authority we have faced several problems with it. As the Directive had not provided clear guidance on the status of the NTS the Hungarian law drew it under the scope of project evaluation. The authority has the right to reject the NTS and oblige the applicant to re-write it as a condition of a favourable decision. In the last 5 years nearly 50% of the NTSs of otherwise accepted applications had to be modified. Whereas the expected benefits and scientific importance are amply emphasized the harms are often ''forgotten'' and the 3R principles are formulated in void general terms e.g. ''we follow

the 3R'', ''apply anaesthesia whenever necessary'', ''always use the minimum number of animals''. These summaries reflect an attitude which consider this document as a tolerance toll to be paid for getting acceptance of the research. On the other hand, writing merely in technical language and wording too much ''scientifically'' may rather be a skill problem than an ethical attitude. Obviously, not every researcher is a talented ''preacher'', and often they need help in learning how to talk about complicated issues in plain lay language (translational task). Professional societies may provide this help by sample NTSs. However, well written NTSs can serve as a communication channel toward the society explaining and justifying the use of animals while simultaneously demonstrating their responsible use and a culture of care (transparency tool).

OE6S3

The ethical importance of transparency of non-technical summaries (NTS)

Weber Tilo
German Animal Welfare Federation – Animal Welfare Academy, Neubiberg, Germany

Abstract
EU member states are required to publish NTS of all applications that they authorise. These summaries must include information on the objectives of the project including the predicted harm, the benefits and the number and types of animals to be used, and compliance with the 3Rs.

In a current study, we reviewed the quality of a representative sample of NTS that were published by the German Federal Institute for Risk Assessment and consistently found inadequate descriptions lacking vital details explaining what is being done to the animals and what harms they might experience as a result. Many also contained complex technical terminology and unjustified reassurances, all contradicting transparency.

Furthermore, we repeatedly noticed references to general statements provided by interest groups who support the use of experimental animals to justify the benefits of the procedures on animals in the application. This is ethically problematic, irresponsible and in breach with the Directive. An objective description of the potential benefits included in NTS is essential for the ethical evaluation. It enables the public to independently draw a personal conclusion whether they consider the authorized animal experiments to be ethically justified.

We call on applicants to take on their responsibility to contribute to an increase of transparency on animal use for scientific purposes as required in the EU Directive and to provide sufficient and objective information in the NTS. Only then the obligations of Directive 2010/63/EU and thus the public demand for increased transparency on animal use for scientific purposes can be fulfilled.

OE6S4

A working group guidance document to improve the language and understanding of non-technical summaries

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Abstract
Non-technical summaries (NTS) are a positive development in improving transparency on animal research to the public, but there are problems in their compilation, accuracy, standardisation and accessibility. The European Animal Research Association (EARA), after discussions with the European Commission, set up a Working Group (WG) to identify opportunities to improve the language used in NTS to make them more understandable for the public. The group had representatives from the user community and experts in animal welfare, communications and private and public biomedical research. The WG submitted the guidance document to the Commission for consideration during its overall work on the revision of the NTS template.

This guidance document offers recommendations to researchers on how to improve the language and understanding of the NTS for the general public. It starts with some indications to researchers on the overall process of producing NTS, and then examines the sections of the original template outlined in the 2013 working document on NTS by the National Competent Authorities for the implementation of Directive 2010/63/EU. Specific recommendations are given on how to write the following: Project title, duration of project, keywords, species and numbers; Objectives; Potential benefits; Adverse effects; Replacement; Reduction; and Refinement. Although the objective of the WG was not to critique the template itself, additional recommendations to the Commission are included concerning the description of the potential purposes of projects. These recommendations, offered to researchers in a direct and concise style, will be presented for each of the sections of the NTS template.

OE7W1

The 10 steps to institutional transparency on animal research

Leech Kirk
European Animal Research Association, London, United Kingdom

Abstract
Since the spring of 2018 the European Animal Research Association (EARA) has been assessing the websites of European institutions that carry out biomedical research using animals. The

results, from the 1,300 websites assessed, reveal that in many countries the research community is still reluctant to provide suitable information for the public on the research it carries out. Just under half (44%) of the EU institutions conducting animal research carry a recognisable statement on their websites explaining their work. On another measure, only a third (33%) of EU websites carry imagery related to animal research. This means that those opposed to animal research are effectively the only source of information the public has. Finally, just half the EU websites (49%) assessed featured a case study on the animal research they support, fund or conduct.

In the digital world, communicating to the public on the importance of animal research via an institution's website is an important way of increasing understanding and awareness of the work of the life sciences sector. This openness allows researchers to place animal research, in context, as a necessary part of biomedical research with benefits to both humans and animals.

In this workshop, we will look at how to establish good practice for a long-term communications strategy, including the 10 steps that any institution needs to take to put in place a robust and effective communications website platform on animal research, and handling crisis communications situations

OE8S1

Performance assessment that links the training and motivation together

Helppi Jussi
Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), Dresden, Germany

Abstract
Laboratory animal programs are being challenged to do more with less, as the economic pressure forces institutions to look more into the cost effectiveness of their operations. For decades this has been an expectation of managers in other industries. Proper management techniques applied in the daily running of animal facilities will lead to better motivation of the workers and more effective operation of the facility. Importantly, significant savings in yearly running costs can be achieved. Empowering animal care staff to take over the responsibility for their own work and to become better informed, more independent, and overall, develop to be a better motivated work force will result in a more effective working culture. We have developed a simple performance assessment chart for the common husbandry and technical skills that we expect caretakers and technicians to perform. The chart is a common effort and assessment by the manager and staff member, thus allowing the staff to be tightly involved in designing and evaluating their own performance enhancement program. The chat itself is tightly intertwined with the weekly training program, and the chart guides both trainees and trainers. Our training program offers every staff member the possibility to function as a trainer, and as a trainee, and the chart helps us to transparently and easily manage the continuous training efforts. Ultimately our performance assessment chart guarantees the staff members' continuous ability to perform the tasks to the highest expectation.

OE8S2

Study compliance liaison process and function

Reinhard Gregory
Office of Animal Welfare, University of Pennsylvania, Philadelphia, United States

Abstract
The directive 2010/63/EU has specific requirements for adequate education and training prior to working with animals and it requires the approval of animal procedures before a study may begin. These prerequisits are very important but for the most part, they are prospective. What happens during the study? Are researcher's skill set up to the task? Is there drift from the approved procedures? How can we provide assurances to the institution and public that we following the rules?

What this presentation will explore is an evolving program of compliance gap identification and real-time performance reviews and correction processes. It has its basis in quality assurance as well as a program in the USA commonly called ''Post Approval Monitoring'' Compliance Liaison is part of both and it is to work with researcher and vivarium staff to identify gaps in compliance and performance and work to their correction. The benefits are multifaceted in assisting the researcher in areas of needed improvement or additional training thus improving their research, it notes documentation gaps and facilitates correction. For the institution, it identifies areas of improvement for training programs and provides assurance of compliance. The personal interactions promotes the culture of care for animals with the researcher. A robust oversight program demonstrates to the government that the institution cares about its animal research programs and identifies problems and promptly addresses them. The presentation will discuss who can perform these tasks, how the reviews are performed and the programs general effecient operation.

OE8S3

Diagnostic tool to assess the health of your animal care program

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[2]*Institutional Animal Care and Use Committee, Emory University, Atlanta, Georgia, United States*

Abstract
Emory University has more than 300 laboratories using animals in biomedical research. The Emory Institutional Animal Care and Use Committee (IACUC) reviews approximately 550 research protocols per year covering a variety of species from birds and fishes to rodents, rabbits, swine and nonhuman primates. The IACUC has the responsibility to review concerns regarding the care and use of animals involved in research and to report issues of non-compliance to regulatory agencies. To achieve that, the Emory IACUC has developed a system for evaluating protocol non-compliance or failure to follow Emory Policy or other regulations and guidelines. The system is used to identify, review, manage and ultimately

prevent any issues of non-compliance. The IACUC uses a point system to evaluate and track non-compliance. Points are tracked on a rolling 12-month basis and are assigned to the Principal Investigator, rather than the individual protocol. The system is designed to determine the level of action to be taken based on the seriousness of an occurrence or its repetition. Using this system, we have been able to detect common problems, identify at-risk investigators in need of additional training, and determine the course of actions to take in a consistent manner. All issues of non-compliance or adverse events are reviewed by a sub-committee, using this system, before being presented to the entire IACUC. The system helps us with our post-approval monitoring (PAM) Program, and has allowed to identify preventive measures that help investigators maintain the highest standards in the care and use of animals.

OE8S4

How to survive a corporate animal welfare audit?

Reinhard Gregory
Office of Animal Welfare, University of Pennsylvania, Philadelphia, United States

Abstract
This presentation will explore the process on how corporations and biotechnology companies review and approve outsourcing (contract) institutions and collaborators for animal research studies. Although each company has their unique processes, there are common themes as well as a general desire to harmonize and even share information. The presenter will review common trends and provide insight on common errors. If the two different institutions are from different countries, how to best present your institution and manage cultural and regulatory differences. The presenter will also touch on animal welfare parts of GLP and GMP audits. The presenter has experience in directing animal welfare due diligence programs for two major pharmaceutical programs and large Biotech Company, and managed over 250 contract sites and preformed over 100 animal welfare audits in 17 countries.

OE9W1

Making openness a reality

Williams Bella[1] and Decelle T.[2]
[1]*Understanding Animal Research, London, United Kingdom*
[2]*Sanofi, Paris, France*

Abstract
Following the launch of the Concordat on Openness on Animal Research in the UK in 2014, and the EARA-led Spanish Transparency agreement on Animal Research in 2016, there has been a move towards greater openness and public-communication by institutions carrying out animal research around the world.

The experience of the UK and other regions has shown that openness is the most effective way to reduce the risk of activism, support public trust and allow effective external scrutiny to improve animal welfare practices, yet moving to greater openness and fostering transparent communication practices in different regions with very different social contexts can present new challenges for the organisations concerned and for the sector as a whole.

In this round-table discussion, six speakers will present their perspectives on openness across regions, in-country and global, considering where openness has been successful, where it has been more challenging and how we can move forward to make openness a reality in more organisations and contexts. Open discussion will be invited following each introductory presentation.

The session will cover the following topics:

- Impacts of the UK Concordat
- Making openness a reality: implementing a transparency agreement
- Understanding barriers to openness
- Taking openness beyond Europe I
- Taking openness beyond Europe II
- Global organisations: corporate messages and collaboration across frontiers

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OE9W2

Towards an 'Openness Statement' for Australia and New Zealand

France Malcolm[1] and Salinsky J.[2]
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[2]*Office of Research Strategy and Integrity, University of Auckland/ Te Whare Wananga o Tamaki Makaurau, Auckland, New Zealand*

Abstract
Despite robust regulatory frameworks supporting laboratory animal care and ethics in both countries, neither Australia nor New Zealand have yet established a formal, nationwide program to promote openness in animal research. This is surprising given the positive responses by the research community to the UK Concordat and similar initiatives in other countries. For several years, however, interest in promoting greater openness has been consistently strong among delegates at conferences of two organisations that directly serve the animal care and ethics communities in our two countries: The Australian and New Zealand Laboratory Animal Association (ANZLAA) and The Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART). Support is currently being established for a joint working party with representation from ANZLAA, ANZCCART, the research community, funders and animal welfare bodies with a view to developing a voluntary 'Openness Statement'. Signatories from both countries would commit to increased transparency and public engagement in their animal research programs. By establishing an agreed set of expectations, it is hoped that this shared approach will result in a more consistent and supportive environment for informing the broader community, nurturing a more constructive discourse on animal research and increasing animal and human health and welfare.

OE10S1

Improving reproducibility and translation of animal research: The European Quality in Preclinical Data consortium

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Abstract
Many data from animal studies cannot be reproduced due to methodological shortcomings, or issues with internal and external validity of research data, with sometimes far-reaching consequences on drug development and translation to humans. With the support of the European Union, a new Innovative Medicines Initiative (IMI) consortium of scientists at leading universities, pharmaceutical companies, contract research organizations and scientific associations was formed, called the European Quality in Preclinical Data (EQIPD) consortium (https://quality-preclinical-data.eu/). Its goal is to investigate the variables that influence the quality of animal study data in drug research and to compare the quality of studies conducted by the pharmaceutical industry as well as academic research, and to develop a fit-for-purpose, preclinical quality management system. We are also working on an online education platform that provides certified training in areas such as optimizing a research design, internal validity, data analysis and standards for reporting. The consortium has 29 participants including private and public research institutions from 8 European countries and the US, and also collaborative partners including AAALAC International and ICLAS providing input on animal care and use matters. Here, we will describe the infrastructure, goals and objectives of EQIPD, and first results.

OE10S2

Severity assessment in animal experimentation – Is that ethical?

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[2]*3R Centre JLU Giessen, Giessen, Germany*
[3]*Messerli Research Institute, Vienna, Austria*

Abstract
To evaluate pain, suffering, distress and lasting harm in animal experiments a severity assessment and classification system has been established in order to promote and harmonize animal welfare across the EU. The definition of harmful procedures is laid down in EU Directive 2010/63, though it leaves a lot of room for interpretation. Severity assessment and classification is one of the main responsibilities of laboratory animal veterinarians. However, in everyday veterinary practice, they are often confronted with inadequately defined requirements and ethical questions are raised, which need to be discussed interdisciplinary.

The Network of Veterinary Ethics, which combines ethics and veterinary medicine, has initiated a discussion in order to clarify morally relevant harms in terms of animal welfare. Currently, the morality of severity assessment is based on the principles of pathocentrism, hence the severity assessment and classification system focuses on negative experiences of animals as a result of painful or distressful procedures. In laboratory animal science this morality does not work as well-being cannot be clearly assessed by humans in all cases. As a consequence, severity classification of procedures and genetically caused phenotypes are not harmonized across Europe. There is an urgent need for improving criteria determining harmful procedures or phenotypes to reach a transparent and consistent understanding of harms inflicted to animals. The moral mandate of veterinarians to reduce harm to animals to an indispensable minimum cannot be fulfilled due to the lack of uniformity. The Network of Veterinary Ethics will present the outcomes of its discussion and possible solutions.

OE10S3

Emotions and ethical decision-making in animal ethics committees

Tjärnström E., Weber E., Hultgren J. and **Röcklinsberg Helena**
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Abstract
Ethical evaluation of projects involving animal testing is mandatory within the EU and other countries. The purpose is to balance the potential harm to the animals with potential benefit to humans, in order to ensure moral standards, scientific validity, and public trust. However, the evaluation process is a complex task that has been subject to criticism with regard to e.g. disparate views on the task, practical difficulties in performing a utilitarian weighting of harm and benefit, and difficulties in ensuring democratic communication structures. To further investigate the decision-making process, we sent out a survey to members of all Swedish Animal Ethics Committees (AECs). We analyzed the role of scientific norms, application of the harm–benefit model, and the role of emotions in the ethical decision-making process.

We found clear differences between member groups in how they perceive the committee work. Researchers and animal laypersons made significantly different ethical judgments, and hold disparate views on which ethical aspects are the most relevant. Laypersons more often felt emotionally engaged in the evaluation, but also that they had less influence. Researchers were more content than laypersons with the functioning of the committees, indicating that the ethical model used suited their preferences better. We argue that the prevailing scientific norms are preventing necessary conditions for sound ethical evaluation consideration by excluding some members from the discourse. In order to secure a democratic and proper ethical evaluation, the expectations of a scientific discourse must be acknowledged, while giving room for different viewpoints.

OE10S4

Aims and achievements of the Dutch National Platform of Animal Welfare Bodies

Salvatori Daniela
AWB-Platform, Dutch National Platform of Animal Welfare Bodies, Denhaag, Netherlands

Abstract
Since the official start in 2016, we now have an association composed of 45 Animal Welfare Bodies (AWBs) from academic and commercial organizations. The major goal is to provide a platform where members share information and find advice (creating ''best practice'' documents) regarding laboratory animal matters. The Platform aims to stimulate discussion and to build a shared view about the interpretation and application of policies and procedures of legislative provisions in monitoring animal welfare in its widest sense. The Platform represents AWBs in discussions and consultation with national authorities and the inspectorate to create broad support from all stakeholders.

Quality of animal experiments and continuing professional development (CPD) are central themes; in fact we have organized several meetings on the importance of experimental (randomisation, blinding) and statistical design. In line with the intentions of the directive 2010/63/EU, we are setting up continuing professional development (CPD) strategies. Together with the Dutch 3Rs Centre we have put together a practical CPD-guidance document for AWB's and have made this available internationally as well. Importantly, we have started a collaboration with the National Committee (NC); the aim is to bring together available codes of practice and best practices and update these where relevant.

The year 2018 was a tough year because we lost our president Harry Blom: his remarkable and international knowledge and amiable personality will be greatly missed. To honour his memory the Platform of AWB's together with the NC will organize a yearly symposium on laboratory animal science topics.

OE10S5

The Dutch foundation for public Information on animal research: SID

Fentener van Vlissingen Martje, Coenen T., van Oosten A., Kluppel A., Visser E. and Frieling W.
SID, Haarlem, Netherlands

Abstract
Introduction: Animal research is of interest to society, firstly, because of the benefits of research for humans, animals and the environment. Secondly, there are ethical issues at stake and animals used for research need to be legally protected. The Dutch foundation for public Information on animal research (SID) provides information about animal research and related issues, and in a compact and accessible way. Recent national developments such as the program for Transition to animal-free research and specific ethical considerations on research aimed at agricultural animal production are also covered.

The SID uses donated funds, and contributions 'in kind', for its activities. The information provided is to be balanced, in support of independent moral choices by individuals. The responsive website www.informatiedierproeven.nl provides a lot of well illustrated documents, background facts and figures, and short films. It is visited by approximately 14,000 unique visitors annually, mostly September – June. Questions submitted by email are always answered, complex ones by experts from the field. Recently a guest lesson program for schools has been launched where volunteers teach school children (aged 10+) and use an interactive tool for anonymous polling and group discussions. Schools evaluate this very positively and the program is being extended depending on the availability of volunteers. SID also functions as a platform for media to reach experts and organizations in the field for interviews and other journalistic productions. SID collaborates internationally with organizations with similar functions.

OE11S1

Emotional dissonance in animal research

Davies Keith
Joint Biological Services, Cardiff University, Cardiff, United Kingdom

Abstract
I will present the primary causes of emotional dissonance and outcomes that emerged from my PhD research, focussing on UK Animal Technologists. Concepts behind the construct of Emotional Labour will be aligned to the tasks and moral dilemmas faced by this workforce in discharging their daily duties. I will also raise awareness of challenges faced with Animal Technologists who may be suffering from some form of emotional dissonance, for example guilt, shame or sadness, but who might have fallen beneath the management radar, prompting the need for a greater emphasis on Emotional Intelligence in management regimes. I will conclude with a brief review of the key task propagating emotional dilemmas faced by those caring for animals – that of euthanasia.

OE11S2

Preventing compassion fatigue through establishing a memory garden

Jameson Tim[1], Redmond J.[2] and Rettew S.[3]
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[2]*Animal Welfare and Comparative Medicine, Covance Research Products, Denver, PA, United States*
[3]*Immunology Services, Covance Research Products, Denver, PA, United States*

Abstract
Compassion fatigue is recognised as a potential outcome from Emotional Labour. Its cause relates to exhaustion from caring for, connecting with, and working with animals. Compassion fatigue varies by person and its effects of can range from mild to

significantly impacting an individual's wellbeing and personal life. Recognising the importance of sharing and listening to colleagues about the potential impact of emotional fatigue a Compassion Fatigue Committee was formed. This presentation explains the wide variety activities and benefits of this initiative. One of these activities, working together on a memory garden, has drawn significant recognition and praise from colleagues, regulators and industry partners.

OE11S3

Emotional labour, responsiveness and animal use

Williams Bella
Understanding Animal Research, London, United Kingdom

Abstract
Those who work in animal facilities are expected by organisations and by the legislation governing use of animals in research to show care in how they use animals. The expectation of a Culture of Care demands that bonding and empathy for the animals is felt not only by the facility care staff, but also others working in the animal facility such as researchers, vets and administrators.

Scientific researchers who bond with their animals undertake emotional labour during their work. Accounts from researchers who trained since caring practices in animal facilities were expressly required in legislation (Directive 2010/63/EU) report that this connection is necessary if they are to write projects and protocols that are sensitive to the experience of the animals. Senior researchers are also affected by their experience of animal death and steps taken to adjust and desensitise to it. While many organisations have an expectation of care, welfare and good-science, few take steps to identify and actively support the emotion-work carried out by all those working in the animal facility. Supporting emotional labour and care among all staff can lead to greater sensitivity, bonding across the workforce and appreciation of animal welfare needs, while reducing stress and staff burn-out.

This presentation will be followed by an interactive discussion session, led by Keith Davies and Bella Williams, to illustrate and consider some of the problems presented by emotional labour in a facility-management context.

OE12W1

National Committees and Animal Welfare Bodies under Directive 2010/63/EU

Louhimies Susanna and Anderson D.B.
Directorate-General for the Environment, European Commission, Brussels, Belgium

Abstract
Within the terms of Directive 2010/63/EU, all Member States are required to establish a National Committee for the protection of animals used for scientific purposes (Article 49) and to ensure that each establishment (user, breeder and supplier) maintains an Animal Welfare Body (Articles 26 & 27). The main functions of the National Committee are to advise competent authorities and animal-welfare bodies on matters dealing with the acquisition, breeding, accommodation, care and use of animals in procedures and ensure sharing of best practice. Animal welfare bodies are expected, within the establishment, to provide advice on the welfare and care of the animals, the application of the Three Rs, review management and operational processes, follow the development and outcome of projects, and advise on rehoming. This is a key structure in developing and maintaining a good Culture of Care and bringing Three Rs to life in the day-to-day activities of the establishment. The Directive review, completed in 2017, identified animal welfare bodies as one of the elements of the Directive that has already started to deliver positive benefits. At the time of the review, however, National Committees were not yet fully developed, but more recently significant progress has been made. The presentation will discuss these developments, remaining challenges and potential solutions to ensure complementary functions of these structures for the benefit of animals and science.

OE12W2

The UK Animals in Science Committee Animal Welfare and Ethical Review Body initiative

Robinson Sally
Member of UK Animals in Science Committee AWERB Subgroup, London, United Kingdom

Abstract
Following Article 49 of EU Directive 2010/63 requires each member state to establish a National Committee. In the UK this is the Animals in Science Committee (ASC) which is an advisory non-departmental public body. One of the functions of the ASC is to advise Animal Welfare and Ethical Review Bodies (AWERB) on sharing good practice within the UK. It is recognised that AWERBs can learn from one another to improve processes for addressing all AWERB functions, ultimately improving the implementation of the 3Rs and Culture of Care more widely than at the individual establishment level. The development of a national AWERB network, based around regional 'Hub' AWERBs, was undertaken to help fulfil that mandate The AWERB Hubs initiative provides a mechanism to facilitate identification of examples of good practice. Such good practice examples might be disseminated to stimulate further development or new approaches to deal with issues of challenges. It is envisaged that dissemination of good practice has the potential to improve efficiency and effectiveness for AWERBs more broadly. The ASC has developed and provided a number of resources to promote and support this initiative. Experience to date has been generally positive and the initiative provides means for the ASC and AWERB Hub chairs to interact and to facilitate two-way communication.

OE12W3

Animal Welfare Bodies in close collaboration with Ethics Committees in Belgium

De Vroey Guy
Laboratory Animal Science and Welfare, JANSSEN RESEARCH & DEVELOPMENT, Beerse, Belgium

Abstract
In Belgium, the Ethics Committees are existing within institutions since the 90's. Their proximity with the researchers and good knowledge of the institutional structures, policies and procedures regarding animal care and use allow an effective dialogue with the principal investigators and biotechnicians to work on a good mutual understanding and to get the ethical viewpoint integrated in the scientific objectives. Trying together to find ways to develop an ethically sound good scientific protocol is the common goal.

The installation of Animal Welfare Bodies, required by the 2010 Directive, is a good way to complement the missing link: getting advice on good practices from people who know how to deal in practice with animals and how to care for them, and to follow-up in the labs the implementation of the approved procedures. A close connection between the Animal Welfare Bodies and the Ethics Committees gives a strong support to the researchers and generates an excellent comprehensive support to develop a culture of care.

With the regionalization of the governmental matters in Belgium, lab animal legislation implementation is at risk of allowing for discrepancies among the regions, but regional committees including representatives from the major academic and private institutions are in contact and the Belgian Council for Laboratory Animal Science generates platforms nationwide for the Ethical Committees and for the Animal Welfare Bodies to exchange best practices and to communicate about harmonization.

OE12W4

Animal Welfare Bodies and Ethic Committees: the French experience

Bruyas Sandryne
Ministry of Agriculture, Paris, France

Abstract
As in all Member States, the requirements to maintain a National Committee for the protection of animals used for scientific purposes and to ensure that each establishment is linked to an ethics committee and establishes an animal welfare body were incorporated in the French legislation in February 2013.

Whereas voluntary ethic committees were already in place in many establishments, the new requirements remained a challenge in others. A national Chart on Ethics in animal experimentation was updated in 2014. Thereafter, the National Committee of Ethical Reflexion on Animal Experimentation has to validate the ''Guide of good practices for the functioning of Ethics Committees'' . Its goal is to harmonize the ethical evaluation by the 125 ethics committees, allowing for a robust and thorough process. In France, Ethics Committees and Animal Welfare Bodies are two separate entities: the first one may be internal or external of the establishment, the second one is necessarily internal.

The roles and responsibilities of Animal Welfare Bodies are numerous, one essential role is to provide practical and up-to-date information on the monitoring of projects and procedures feedback. These Bodies have allowed/allow for progress on implementation of the 3Rs within projects; they also enable better communication between all parties involved and thus ensure a good culture of care. The number of animal welfare bodies and ethics committees can be a difficulty for a good communication with the National Committee. This presentation will provide an overview of the challenges encountered and discuss possible improvements.

OE12W5

Networking to promote a Culture of Care

Bertelsen Thomas
Animal Bioethics, Novo Nordisk A/S, Maaloev, Denmark

Abstract
The concept of Culture of Care is stated in Directive 2010/63/EU – ''The [AWB] body should also . . . foster a climate of care'' – and is further expanded in EC guidance document ''A working document on Animal Welfare Bodies and National Committees'' where it states that the AWBs play a fundamental role in establishing and maintaining an appropriate culture of care.

Used the right way, a committed culture of care is a strong support to an animal centric approach of working with the 3 Rs. Culture – defined as the way we think and behave – in terms of animal welfare can only become an effective enabler if the AWB is reaching out and interacting with a combination of different stakeholders and operators.

One way of reaching out and interacting is by networking, and after the session on culture of care at the 2016 FELASA congress in Brussels, the international network was introduced and launched.

The network quickly agreed on a working concept including amongst other matters: to go beyond simply having animal facilities that meet minimum requirements of the legislation, an appropriate behaviour and attitude towards animal research, a pro-active approach towards improving standards, all voices and concerns at all levels throughout the organisation are heard and dealt with positively.

Today the network has 38 members from 16 different countries representing – apart from AWB members with 'hands-on' animal welfare responsibilities – members from national authorities, NGOs and also members with communication skills. The presentation will update on the network's progress, achievements and challenges.

OE13S1

Reporting requirements under Directive 2010/63/EU for Genetically Altered Animals (GAAs)

Anderson David and Louhimies S.
European Commission, DG Environment, Brussels, Belgium

Abstract
Directive 2010/63/EU requires that Member States shall collect and make publicly available information of the use of animals in procedures on an annual basis. Commission Implementing Decision (2012/707/EU) provides the detail on the information to be provided, including for GAAs. GAAs shall be reported i. when used in the creation of a new line, ii. when used for the maintenance of an established line with an intended, and exhibited harmful phenotype, or iii. when used in other (scientific) procedures (i.e. not for the creation or for the maintenance of a line). Additional guidance was provided in the Working Document on GAAs endorsed by the National Contact Points in 2013. As part of the five-year Implementation Report due to be published later in 2019, additional information is collected on GAAs not otherwise reported in the annual statistics. Furthermore, information on methods to obtain tissue for genotyping will be provided. Feedback, in particular during the Directive Review, suggested that differing approaches were being taken to regulation and reporting of GAAs. This risks impacting on a level playing field across the EU for the scientific community, in some cases undermining animal welfare, and affecting the consistency of statistical reporting of animal use. As a consequence, an Expert Working Group was convened in June 2018 to review these concerns and the appropriateness of the present guidance in view of the development and increasing use of gene editing technologies. The presentation will provide an update on these deliberations.

OE13S2

How ought we to recognise and classify a genetic defect?

Morton David
University of Birmingham, Birmingham, United Kingdom

Abstract
Current classifications of harmful defects in genetically altered/modified animals rely on human observations of 'abnormal' clinical signs. This is a helpful, legitimate and valid approach but, at present, it measures the ability of humans to be observant, and makes no allowance for the ability of animals to hide or cope with any defect. A genetic, or indeed experimentally induced, defect may not simply be an overt change in physical appearance or behaviour in an animal, but may reflect subtle mental adaptations. It is also a failure of such animals to realise their genetic potential. The ability of animals to 'survive' in a laboratory environment is only part of this 'anthropo-centric' story, as we have no insight into what natural instincts of an animal to survive might have to be employed to cope with any defect. However, what we can often reasonably deduce is that such animals would be unlikely to survive in the environmental niche in which they have evolved compared with non-modified beings. They will lack a fitness to survive and their biological integrity is restricted, and thus their welfare is fundamentally impaired. Non-harmful genetic defects such as congenital hearing loss or blindness, etc are difficult to assess when animals are in protected and caring environments, but they should not be classified as being 'non-harmful'. I will discuss systems of assessing these types of animals, and suggest a different level of severity classification.

OE13S3

GA animals' welfare assessment: An interdisciplinary approach

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Abstract
Working with genetically altered (GA) animals requires special attention to phenotype-associated welfare issues. A description of the phenotypic characteristics serves as the fundamental basis for welfare assessment and severity classification. In terms of animal welfare, the main objectives are determining potentially harmful phenotypes, implementing refinement strategies to minimize suffering of lines affected by a mutation, and communicating severity levels of such animals used in scientific research to the general public.

Experience of recent years show that classification of the phenotypic impact of genetic modifications differ widely between institutions and surveyed experts across Europe. The aim of the study is to assess whether the phenotyping data collected by high throughput phenotyping platforms can be used to review and refine current criteria for welfare assessment and severity classification of GA animals.

Extensive phenotypic data of lines generated by the International Mouse Phenotyping Consortium (IMPC) are available online and serve as a valuable source of information. Lines characterized by significant phenotype associations and annotations in one or more of the applied phenotyping protocols are randomly selected for review. In addition, observed animal welfare relevant information are matched to the IMPC results and reviewed accordingly.

OE13S4

The International Society for Transgenic Technologies (ISTT) – Fostering exchange

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Abstract
The International Society for Transgenic Technologies (ISTT), founded in 2006, is a vibrant community of scientists, technicians and graduate students with currently more than 700 members in 37 countries. The ISTT brings together all those working in animal transgenesis. Our declared goals are to foster and encourage

knowledge generation, discussion, training and education, and to share technologies and specific research used for the genetic modification of animals.

We act as meeting point and debate forum for all professionals and students in the field. On our website (www.transtechsociety.org), members find plenty of information such as selected talks, posters, methods, posts and messages. The ISTT organizes and promotes courses, seminars and every 18 months an international 'TT meeting'. Sharing best practices and techniques are aimed to refine experimental techniques and to reduce animal numbers in alignment with the 3R principles.

Furthermore, we associate with other national and international societies with similar aims, such as FELASA, and provide expert advice and guidance to local, national and international bodies regarding scientific, technical, or other aspects of animal transgenesis.

OE14S1

Revealing the full picture: How continous and comprehensive phenotyping can inform severity assessment

Wells Sara
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Abstract
Every year many thousands of new genetically altered (GA) mouse strains are added to the catalogues of resources available to researchers investigating gene function and the genetic influences of disease. As the proliferation of different genetic models increases, so does the depth and accuracy of which we can measure their phenotypes. Recent advances in home gage monitoring, telemetry and biomarker assays, to name a few, are giving us a deeper understanding of the actions of genes and how they may cause deficits in GA mice. Unsurprisingly, many of these phenotypes are not visible from a simple inspection of the animal. Behavioural tests may only examine a snap-shot in time, in an anxiety-inducing environment which may lead to the assessment missing sporadic behaviours or the animal masking a subtle deficit. Similarly, it is not obvious from a cage-side assessment during the light phase whether an animal has raised levels of metabolites or indeed has sensory deficits such as hearing-loss or blindness.

How to capture, interpret and report these observations so that the true extent of any harm cause by a genetic modification can be assessed is a challenge we must overcome. With intense and sophisticated analysis of the phenotypes we will undoubtably be able to increase the accuracy of harm assessment and better define the care required for individual GA lines.

OE14S2

Evaluation of ultrasonic vocalization as a tool for assessing welfare state in mice

Medina Camilo
Max Planck Institute for Evolutionary Biology, Plön, Germany

Abstract
Directive 2010/63/EU with strong fundaments in the 3R's principles introduced in its annex VIII a severity classification of procedures determined by the degree of pain, suffering, distress or lasting harm expected to be experienced by an individual animal during the procedure. Every project, depending on its level of severity it will be subject to different restrictions.

As ensuring the best care/ welfare possible became an essential part of every project, this research is oriented to evaluate the possibility of employing mice Ultrasonic Vocalizations as a tool to assess the level of impact over the animal's wellbeing (and eventually the level of severity) of some common practices in the daily work with mice.

Changes in frequency and amplitude in mouse ultrasonic vocalizations can be an indicator of alterations in emotional state as well as an indicator of reward or distress. Some studies also propose that pain can be socially transferred between familiar mice due to empathic responses. In this context, the vocalization can provide valuable information about changes in the emotional state of certain individuals or group of animals becoming a potential welfare indicator.

We believe that a better understanding of the context and characteristics of the mice Ultrasonic Vocalization can improve our comprehension regarding the impact of some common procedures. Eventually, a deeper knowledge of mice Ultrasonic Vocalization can contribute to develop better and less invasive methods to assess early detection o of distress in mice ensuring a better categorization in the level of severity of a given intervention.

OE14S3

Severity assessment in zebrafish

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Abstract
According to EU and national regulations, a project authorization requires a classification of applied and performed procedures according to severity based on estimated or observed levels of pain, suffering, distress and lasting harm inflicted to the animals. This assessment results in a definition of a procedure as non-recovery, mild, moderate, or severe. While non-recovery is usually not debated, the other classifications are difficult to apply in non-mammalian species. In zebrafish, this discussion becomes prominent since pain perception and the resulting physiological effects are not well understood. Especially, the high regenerative capacity of zebrafish often leads to the assumption that a procedure is not harmful. Besides that, experimenters sometimes underestimate physiological effects on the well-being of the animal. They are not always recognizing that also short-term impact without lasting effects must classify a procedure as harmful. Also in genetically modified lines, constant effects of the genetic mutation may lead to burden in the animal. I will present some examples of procedures or harmful phenotypes occurring in research facilities and what effects may result from them. What parameters may help to evaluate the level of burden and by this defining it as ''mild'', ''moderate'', or ''severe'' is also a matter of discussion.

OE14S4

Exchanging information on genetically altered animal welfare assessment: From theory to practice

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Abstract
The recombinant DNA technology has provided tools to modify the genome of an animal, either by knocking out genes or by overexpressing new genes that are linked to important metabolic pathways in health or diseases. CRISPR genome editing technology is also considered as a lot of promising technic leading to new translational paths and also to unexpected mutations. Despite its deficiencies, it has the potential to advance biological understanding. The easy and costless way to generate and use animal models in biomedical research raises serious concerns about the welfare and the quality of life of those animals. Welfare assessment of genetically altered animals is a long-term process that demands responsibility and covers the generation, production, maintenance and finally the use of those animals. Moreover it is equally important to disseminate this information to other scientists especially when these animals are transferred between institutions in different parts of the world. Although academic institutions already use different templates, there is a general belief that these should be harmonized and completed with all the necessary information. That is why the European Commission has already established an expert-working group aiming to create a reporting system/template, which will facilitate the dissemination of the necessary information and minimize the risk of misreporting. Contributing to this effort, the aim of this presentation is to introduce different reporting systems that are used in practice and how these are implemented. Comparing the usefulness of those systems, different examples with mainly harmful genetically altered strains will be used.

OE15S1

Pharmaceutical company reflections on 'Culture of Care' when using animals in scientific research

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[5]*EFPIA, Brussels, Belgium*

Abstract
The term 'Culture of Care' is used in many contexts across different regions and organisations but rarely with any defined indicators to support working practice. The European Federation of Pharmaceutical Industries and Associations (EFPIA) Research and Animal Welfare (RAW) group members reflected on the concept of a Culture of Care and on differences in its understanding and application across European companies. EFPIA's RAW group have developed a framework to help organizations identify gaps or potential areas for improvement in support of a positive Culture of Care.

The framework is a tool that identifies six areas of focus for Culture of Care: corporate values; strategic approach at establishment level; implementation structures; staff; animals and openness. It is intended as an aid for continuous improvement, highlighting where indicators of good practice are present. We expect it to provide points of reflection and ideas for those looking to implement Culture of Care in a structured way, while facilitating a professional and strategic approach. To prevent it supporting a 'tick-box' exercise, the framework must not be used as an auditing tool, but as a starting point for consideration and discussion about how care manifests within the context and constraints of individual establishments.

A Culture of Care is one that is dynamic and continuously evolving, and we expect that even organizations with an established Culture of Care will benefit from reflecting on indicators drawn from across organisations to support further development.

OE15S2

Quantification of the Culture of Care: Development of a corporate key performance indicator

Decelle Thierry and Turkmenian O.
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Abstract
The culture of care has been a component of the policy of Sanofi on the Protection of Animals for more than 15 years. Beyond the formal commitment to embrace a culture of care, there was no system in place to assess the effectiveness of a corporate commitment and no indicator of performance.

The culture of care goes beyond the compliance to regulations. It is related to the attitude of the personnel and the benevolence towards laboratory animals. The Culture of Care aims at improving the relationship between the professional and the animal, to establish a symbiotic relationship between them and to fully consider animals as sentient beings. Therefore the individual skills which are key include responsibility, empathy, openness, engagement, collaborative work, proactivity. However, the attitude of individuals is not sufficient to implement an efficient culture of care program; the institution, through its values and the leadership of the management, represents a keystone of the program. Culture of Care represents a mix of institutional and personal commitments.

A global questionnaire with scoring system has been developed and sent to 12 Sanofi sites where animals are used. The target population for the survey was animal professionals, technologists, scientists, veterinarians, management line, site direction, animal ethics committee and administrative support. The topics include regulation, oversight bodies, communication, daily job, recognition, animal welfare, culture of care. The talk is to present the approach and the outcomes of the survey and the future steps to strengthen the Culture of Care program.

OE15S3

Culture of Care – Time to switch from words to action

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European Commission, Brussels, Belgium

Abstract
Culture of Care is a central theme within Directive 2010/63/EU *inter alia* through the requirements for continuous application of the Three Rs, and for education, training and competence of staff. The Directive recognises that animal welfare considerations should be given the highest priority in all animal care and use practices. To enable this, each establishment should have an Animal Welfare Body. One of the tasks of the Animal Welfare Bodies is to foster a climate of care.

The theme of Culture and Care is further elaborated in the guidance developed by Member States and stakeholders to facilitate the implementation of the Directive. Culture of Care is featured in the guidance on Animal Welfare Bodies and National Committees, Education Framework, and Inspections and Enforcement.

However, simply meeting the legislative requirements will not ensure appropriate welfare, care and use practices. Although Culture of Care can be anchored in legislation and supported by guidance, it can only be achieved through individual commitment which itself can only be enabled through institutional support. Most of the roles involved directly or indirectly in animal use and care, including and beyond that of Animal Welfare Body, play a part in building the desired Culture of Care. It is time to switch from words to action with concrete tools to help assess and address Culture of Care.

OE16S1

Development of learning outcomes and assessment tools to harmonise competence assessment throughout the EU

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Abstract
Education and Training activities in the EU remain the competence of individual Member States. As a result, E&T requirements are not always clear for the staff/researchers who want to move from one MS to another to work with laboratory animals; similarly it is difficult for authorities to assess competence claims acquired outside of the MS in question when judging compliance with national E&T requirements. The Education and Training Platform for Laboratory Animal Science (**ETPLAS**) pools together three key players: Member State authorities, course providers and course accreditors, as a unique one-stop-shop in the EU. **ETPLAS** has recently been awarded an EU Grant (n°07.027741/2018/794340/SUB/ENV.B2) for a Pilot project entitled ''Promoting alternatives to animal testing through accessible and harmonised education & training''. The key aim of **ETPLAS**, through this grant, is the provision of information and tools for the delivery and assessment of high quality laboratory animal science training in Europe. The Action has 5 key Activities to enable this objective and each of these Activities will be delivered through working groups with representatives of stakeholders from the LAS community such as course providers and members of accrediting bodies. An update of what this entails will be provided. We hope that through these activities the ETPLAS portal will be elevated to a new level with concrete, practical tools that will be of benefit to everyone involved; from stakeholders of the LAS Community to trainees, trainers, accrediting bodies, named persons for education and competence, and MS authorities.

OE16S2

E-learning resources to support training for project evaluation and project and procedure design

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Abstract
EU Directive 2010/63 requires appropriate training of those with responsibilities for the use of animals in research. Implementation of training of those carrying out procedures has been undertaken in most, if not all, member states. Training of those responsible for the design of projects and procedures shows greater variation, and training in the Project Evaluation process has yet to be established in some member states.

Developing such training is challenging and resource intensive. To provide resources to support all those involved in the process, and to encourage a consisten approach amongst member states, the Commission is supporting the development of E-learning programs for Modules EU10, 11 and 25. This initiative will also develop learning outcomes for severity assessment, and the production of an additional e-learning module to meet these learning outcomes.

Such training is an important element in meeting some of the key the goals of EU Directive 2010/63: Ensuring that project applications contain all the necessary information, ensuring that all three ''Rs'' are fully incorporated into the design and evaluation of such studies, promoting consistency in outcomes of harm-benefit analysis.

We have recently commenced work on this project, and aim to produce a series of modules to meet these goals. We aim to present the material in ways that will be useful to bioscientists, veterinarians, laboratory science and technology specialists, and lay persons. The modules will be freely available via a platform managed by ETPLAS, and should be available at the end of 2020.

OE16S3

Promoting the uptake of alternatives to animal testing through the development of eLearning tools

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[5]*Swiss 3R Competence Centre, Bern, Switzerland*

Abstract
In order to further promote the implementation of Directive 2010/63/EU, the European Commission issued calls for a number of related projects last year. One of these projects is aimed at facilitating the uptake of non-animal alternatives by developing two e-learning modules. The contract for this project was awarded to a consortium consisting of SYRCLE, the Swiss 3R Competence Centre, Institute for In Vitro Sciences, Pharma Launcher and Ecorys UK. This consortium will develop two modules, i.e., one e-learning module focussed on searching for existing non-animal alternatives (including systematic reviews) and one module targeted at researchers who want to develop reliable and relevant non-animal alternatives for regulatory use taking into account Good In Vitro Method Practices (GIVIMP). The quality of the developed modules will be assessed by external review groups. The learning outcomes will be presented as well as the design of the assignments through which these outcomes will be realised.

OE16S4

Advancing 3Rs' education and training under a European Parliament pilot study

Holloway Marcelle
EU Reference Laboratory for alternatives to animal testing (EURL ECVAM), European Commission, Ispra, Italy

Abstract
In accordance with Article 48 and Annex VII of Directive 2010/63/EU on the protection of animals used for scientific purposes, the JRC's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) provides support to develop, validate and promote alternatives to animal procedures in the area of regulatory testing and in basic and applied research.

Recent EURL ECVAM studies have shown that although much Three Rs knowledge exists, its sharing could be improved especially between different fields of expertise through better coordination, communication and outreach, and by more emphasis on targeted education and training initiatives.

With this in mind and benefitting from funding made available under a European Parliament Pilot Project, which aims to promote alternatives to animal testing including the application of the Three Rs, EURL ECVAM launched a project to investigate the feasibility of including the Three Rs in educational curricula. The project focuses on high school, university and early career education levels and will produce the following: an outline approach and specification for guidance that informs decision-makers in educational organisations to facilitate the incorporation of the Three Rs into their programme and curricula; specifications for the development of several educational resources that will promote the Three Rs at the three levels of education; and the production of some practical resources that can be used in high schools, universities and continuing education programmes. In addition to this work, EURL ECVAM is engaging in other initiatives to boost education and training in this area.

OE17S1

Comparison of university animal resource/research programmes in the European Union and the United States

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Abstract
In a collaborative study between the League of European Research Universities and Yale University, the Yale Animal Resource Cost and Benchmarking survey© was modified to capture similar thematic information in European universities. Universities from 11 European countries participated. 1/3 of these have one single centralized animal care and use programme and for 1/3 of the institutions the Designated Veterinarian is the Programme Director. Like US programmes, almost all EU programmes include mice and rats, but fewer EU programmes have monkeys and dogs, and EU-animal facilities have about an equal amount of housing and procedure space, while US-facilities tend to have twice as much housing as procedure space. Per diem rates have similar compositions between EU and US programmes, with a little more than 50% covering salary and fringe, followed by supplies (~25%), facility costs (~10%) and other expenses (~15%). Unlike some US-programmes, the EU-programmes tend not to over-recover mouse care costs, but ~60% of both US and EU-programmes under-recover mouse care costs. On average EU-programmes have a small positive net-operating balance, while US-programmes average large deficits. In EU-programmes less than 50% of Institutions automatically cover the animal programme deficit, while almost 100% of such deficits in US-programmes are covered by the Institution. In setting per diem rates, EU-programmes rely more on cost accounting, care more about competitive rates and are less influenced by Animal-User-Groups than US-programmes. Outsourced services are similar, with virology and serology being most frequently outsourced.

OE17S2

Meeting the challenges of animal research in a global context

Turner Ann
American Association for Laboratory Animal Science, Memphis, United States

Abstract
Establishing and maintaining a culture of care in the laboratory animal science arena is seen as an overarching challenge worldwide. Conducting research involving animal models is complex and involves many challenges regardless of the geographic location; however, some challenges are more acute in developing countiries, while others are more widespread in countries with established research traditions. Resource allocation, including funding and personnel as well as animal procurement, is a challenge shared by all those who work in the research arena. However, challenges inherent to resource allocation vary by geographic location. Those who work in biomedical research face the challenges of welfare and care for the animals, fulfilling regulatory requirements, combating animal extremists, informing the public, training of personnel, accommodating diverse languages and cultural mores, and ensuring good practices and standards. Using input from an informal panel of professionals working in the global laboratory animal science community, the similarities and differences in the challenges and the ways those challenges are met will be presented and discussed. Resources of the American Association for Laboratory Animal Science (AALAS) will be highlighted as examples of how a professional association helps research institutions meet these challenges.

OE17S3

The tyranny of distance: Challenges delivering quality animal care in Australia and New Zealand

Collins Kiri and Tatarinoff V.
Australian and New Zealand Laboratory Animal Association, Sydney, Australia

Abstract
In recent years Australia and New Zealand have placed a significant focus in health and medical research spending which has led to a culture of innovation and research excellence. These priorities and subsequent research successes have come with unique challenges for our countries infrastructure and regulatory frame works. This talk explores some of these challenges and how the Australian and New Zealand Laboratory Animal Association (ANZLAA) as a supporting organisation interacts with our research community. Through supporting a range of activities, our association aims to promote communication, informed discussion and continuing education among its members as well as the broader community in pursuit of improved animal welfare.

OE17S4

Moving from crisis management to proactive communication of animal research

Zeller Rolf
President, International Basel Declaration Society, Zurich, Switzerland

Abstract
The Basel Declaration Society (BDS) is a grass-root NGO dedicated to promoting ethically responsible animal research and open communication concerning all aspects of modern life science research that involves animal studies. As part of this mission, the BDS is interacting with other organizations to organize bi-annual international conferences that provide stakeholders with a platform to discuss and network. In contrast to the research community, the opponents of animal research are internationally well connected and funded allowing them to organize powerful and internationally coordinated campaigns against animal research. Recently there have been positive efforts by researchers and institutions to engage more in proactive and open communication. However, many remain reluctant to transparency with the exception of acute crisis situations. Furthermore, most of the proactive communication on animal research relies on the classical media and/or lacks an engaging dialogue, which carries the risk of largely ''preaching to the converted''. As animal research is viewed critical by a significant fraction of the population, it is very important to develop communication strategies that are providing fact-based information in an attractive and interactive manner. The use of social and new media has to be a cornerstone of any such communication strategy. It is very important to reach out to the young generation as they make up the next generation of leaders – they must have access to the facts that allow them to understand why research using animal models remains at the core life sciences to the benefit of society also in the 21st century.

OF1W

FELASA Workshop on Classification and Reporting of Severity – Introductory session

Anderson David, Smith D. and Degryse A.
FELASA Severity Classification and Reporting Workshop core trainers

Abstract
The inclusion a severity classification system within Directive 2010/63/EU provides an opportunity to improve the quality of science and animal welfare through prospective review of research proposals, and, by the inclusion of the actual severity experienced by each animal during a procedure in the Statistical Reporting of animal use, to allow for greater transparency and thus promote improved public confidence in the use of animals in research. Over time, these publications may contribute to trends in refinement. This workshop will deliver an introduction to these requirements using a number of worked examples. The first of these workshops was held at FELASA Congress in Brussels in 2016, and has since then been successfully presented on more than twenty occasions throughout EU.

OF2W; OF3W; OF4W

Classification and Reporting of Severity Workshop – Dealing with complex, challenging and uncommon Models

Anderson David, Degryse A. and Smith D.
FELASA Severity Classification and Reporting Workshop core trainers

Abstract
This workshop is intended to explore further some of the issues with regard to severity classification and reporting of procedures, raised during the introductory workshops held over the last few years. It will moreover include the challenges confronted with when dealing with complex studies using, for example, Genetically Altered Animals (GAAs), including mice and zebra fish, reporting actual severity in GAA breeding colonies and immature forms and taking into account the cumulative severity animals may experience. A similar format to that used in previous Workshops will be adopted, but new examples will be utilised, and some time will be allocated to discussion of problems raised by attendees. It is expected that participants will have previously attended one of the introductory workshops held across EU in last few years.

OF5

Classification and Reporting of Severity – Interactive Q&A session

Anderson David, Degryse A. and Smith D.
FELASA Severity Classification and Reporting Workshop core trainers

Abstract
This informal session is intended for new attendees, trainers as well as attendees to previous workshops as an opportunity to raise experiences or difficulties with classifying, assessing or reporting the severity of procedures within the framework of Directive 2010/63/EU.

Questions or problematic casework can be submitted in advance. Attendees will be invited to present their own casework and an experienced panel will be available to offer advice and guidance.

The session will provide an opportunity to identify and discuss issues which may still be causing confusion and where further guidance/clarity is needed. By sharing information and experiences, an improved consistency of approach will be developed, which can only be of benefit to animal welfare, science and the scientific community.

OF6S1

FELASA Woking Group on Severity Classification – The problem of fish

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Abstract
The European Directive 2010/63/EU requires the prospective severity classification of procedures when applying for a project authorization and the actual severity of procedures to be recorded on completion of procedures. The term ''severity'' encompasses pain, suffering, distress and lasting harm. A collection of common procedures listed in Annex VIII provides examples for estimating severity. Unfortunately, the listed procedures are common in rodents and other mammalian species, but of limited value for aquatic vertebrates, despite zebrafish being the second or third most used species of laboratory animals. A new FELASA Workgroup has been set up to help fill this gap by (1) identifying common procedures performed in zebrafish, (2) trying to list scientific evidence for the possible harm caused to zebrafish as a result of those procedures, and (3) providing guidance and examples for the severity assessment process. Also, (4) the assessment must include the whole life cycle in captivity and guidance will be developed accordingly to developmental stage/phase. Being ectotherms, aquatic animals' physiology fluctuates in relation to external factors to a greater extent than rodents and other mammals and the difference between procedural and non-procedural effects is potentially greater than in other species. As a first step, the Working Group has started a European and worldwide survey in zebrafish laboratories to identify common procedures and how the labs deal with the severity classification to get an overview. Results and further steps will be presented and discussed.

OF6S2

Prospective severity classification of scientific procedures in cephalopods: Outcomes from a COST-Action FA1301 Survey

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[9]*Comparative Medicine Unit, Trinity College, Dublin, Ireland*
[10]*Research, Development and Innovation, Association for Cephalopod Research 'CephRes', Napoli, Italy*

Abstract

Cephalopods are the only invertebrates included in Directive 2010/63/EU; guidance on prospective assessment of severity of procedures for these animals is lacking. To fill this gap we carried out a web-based survey among the scientific community represented by the COST Action FA1301-Cephs*In*Action. 50 mini-scenarios, covering a range of procedures in several cephalopod species at different life-stages inspired by published papers were included in the survey. Responses (59 people, 15 countries) allowed allocation of scenarios to a severity classification; unable to decide (UTD) was also a possible response. Overall, UTD was only $7.0 \pm 0.6\%$ of responses and did not affect the overall severity classification. We will present the outcomes of the analysis of the survey showing that: **i.** consensus on non-recovery procedures was reached consistently; **ii.** Scenarios describing procedures above the 'lower threshold' for regulation were also identified; **iii**.severity classification based on different species (e.g., cuttlefish *vs*.octopus) was reliable and dependent on potentially more harmful interventions linked to species-specific physiological requirements. The COST Action FA1301 survey data provides examples of severity classification of procedures on live cephalopods and is resource for researchers, project assessors and regulators. This web–based, scenario approach to prospective assessment may be applicable to other regulated species and also provides a useful educational tool.

OF6S3

Refinements in disease modelling of zebrafish

Allen Claire

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Abstract

The success of zebrafish as a model organism in medical research has led to a significant increase in the use of this tropical fish beyond its' point of protection. Initially, this fish was famed for having transparent embryos and being a low neurophysiological model, which was good for initial 3R justifications within projects. Since 2014, the scope of many investigations has changed and a shift towards work on legally protected stages has occurred. Furthermore, we are observing increasing complexity and sometimes severity in these procedures. At our facility, we have managed over 200 Individual Study Plans (ISPs) that primarily detail larval stage experiments and account for the use of thousands of zebrafish. We can categorise the protocols that appear on these ISPs into 19 distinct areas ranging from phenotypic analysis and drug testing, to tumour development and behavioural conditioning. We have carefully managed this diverse range of experiments with consideration for ethics and how the 3Rs can be applied to zebrafish. We have begun to assess many parameters, welfare considerations and experimental designs and try to help maximise the power to obtain a scientifically valid result, which is reproducible. We want to share our knowledge and encourage animal researchers to spend more time thinking about these crucial aspects of experimental design to yield more robust, reliable and importantly reproducible animal studies.

OF6S4

Identification of individual zebrafish: A refined protocol for VIE tagging whilst considering animal welfare

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Abstract

Zebrafish are an important model system for scientific and medical research. Despite this, marking zebrafish for individual identification purposes is not commonplace. In other fish species, visible implant elastomer (VIE) tagging is used as a successful identification method but lacks important details regarding fish welfare. We highlight previously unconsidered animal welfare issues through long-term observations of survival rate, tag retention, and tag colour on different populations and age-groups of zebrafish; and introduce an improved VIE tagging protocol. This improved protocol was developed and compared with original tagging procedures and associated negative effects, following animal welfare concepts described in the Three Rs principles, focusing on Refinement. We describe a novel protocol using lidocaine solution as an analgesic and post-tagging treatments with two healing agent to improve the wound healing. The information from this study will be beneficial for the zebrafish research community as a guideline for implementing VIE tagging as a successful identification tool when differentiating between genetic lines, families, or individuals. And will also be beneficial for the whole fish biology community when considering important animal welfare questions in the future if they are using identification techniques which could be considered as potentially noxious stimuli for fish.

OK1

Alternative methods: Myths and reality in a global rapidly-changing landscape

Dal Negro Gianni
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Abstract
In R&D processes, to increase the success rate of new products, predictive capabilities need to be improved for more successful decision-making. These improvements entail the identification of new biological markers as well as new human-relevant tools to assess the efficacy, safety and quality of products. The global rapidly changing landscape makes it increasingly urgent to address gaps in traditional R&D processes, where laboratory animals are used with different success rate. Stakeholders increasingly demand evidence of value, safety and increased cost-benefit ratio of new products. Another important component of the changing landscape is the increasing pressure by the Society and policy makers towards phasing out laboratory animals.

Despite massive investments by the Industry and the European Union in finding non-animal solutions to hazard identification and risk assessment, the limited number of so-called alternative methods formally validated cannot fully replace animals in the regulatory space. However, in the last decade, some important advances in *in vitro* modelling have been achieved. Complex architecture with different cell populations challenged with biochemical and mechanical stimuli, micro-fluidic components, and the integration with analytical devices are some of the advances towards an increasing human relevance and predictive capability. Nevertheless, these technical solutions still have gaps that need to be closed; these gaps are the future challenges for model developers.

Although newest non-animal technologies promise to deliver game changer solutions in R&D processes, they are still in their infancy; this makes the estimation of a possible timeframe for laboratory animal phasing out currently unrealistic.

OK2

Gut feelings about the brain: Rodent models of the Microbiome-Gut-Brain axis

Cryan John
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Abstract
There is a growing recognition of the importance of the commensal intestinal microbiota in the development and later function of the central nervous system. Developmentally, a variety of factors can impact the microbiota in early life including mode of birth delivery, antibiotic exposure, mode of nutritional provision, infection, stress as well as host genetics.

Research using germ-free mice has provided some of the most persuasive evidence for a role of these bacteria in gut-brain signalling. Key findings show that the microbiota is necessary for normal stress responsivity, anxiety-like behaviours, sociability, and cognition. Furthermore, the microbiota maintains central nervous system homeostasis by regulating immune function and blood brain barrier integrity. Studies have also found that the gut microbiota influences neurotransmitter, synaptic, and neurotrophic signalling systems and neurogenesis.

The principle advantage of the germ-free mouse model is in proof-of-principle studies and that a complete microbiota or defined consortiums of bacteria can be introduced at various developmental time points. Alternatives and complementary strategies to the germ-free model include antibiotic treatment to create microbiota-deficient animals at distinct time points across the lifespan. Humanisation strategies are also being employed whereby microbiome is transplanted from humans into germ free mice to address causal aspects of microbiome in shaping behaviour.

Increasing our understanding of the husbandry factors that influence microbiota composition in early life and across the lifespan in laboratory animals will be crucial in increasing reproducibility in the microbiome field and fully exploiting the microbiome for therapeutic strategies for brain disorders.

OK3

Microbiological standardisation and health monitoring of laboratory rodents – Importance and aims

Nicklas Werner
formerly: German Cancer Research Centre, Heidelberg, Germany

Abstract
Health monitoring of laboratory animals originally aimed at detecting causes of clinical disease and death during experimentation. This aim changed, as most agents which have a significant potential to induce clinical disease were eradicated. Nowadays, the main objective of health monitoring is detection or exclusion of a broad panel of unwanted agents that may have impact on research results. Clinical disease due to infections has become rare in laboratory rodents but health monitoring has become increasingly important due to the worldwide exchange of genetically modified rodents. It has been the merit of FELASA to establish the first working group for the health monitoring of laboratory rodents already in 1989 to develop recommendations for uniform health monitoring procedures and a uniform health monitoring format for European countries. Largely standardised health reports facilitate evaluation of the infectious status and thus help to reduce the risk of introducing infectious agents into an animal facility by animals and samples. Sophisticated animal models and testing procedures have shown that not only pathogenic and other unwanted agents may have impact on results from animal experimentation but that also the autochthonic flora must be considered as a research variable. The composition of the microbiota has been shown to be an important factor that can modify research results, and it will therefore become necessary in future to test also for bacteria that are important to accomplish normal physiological functions in laboratory animals.

OWE1

Predictive models for assessment of anti-tumor efficacy of innovative drugs

Leuraud Pascal
Research, Xentech, Evry, France

Abstract
A review of PDX models available for cancer research. The session will review various mouse strains, their differences, and how their characteristics impact research. The speaker will provide tips to researchers to help with in vivo research success. Also, it will cover how PDX models can be used for the development of precision medicine.

OWE2

Challenges and best practises for cage reprocessing in your biomedical research facility

Larsson Joakim, Hohner R. and Nilsson M.
Getinge, Gothenburg, Sweden

Abstract
The integrity of scientific data produced in biomedical research facilities is of paramount importance to humanity. Regular cage cleaning, sterilization, and animal bedding replacement are vital for animal welfare and contamination control in biomedical research facilities. The objective of this session is therefore to give you an answer, by an interactive discussion, of *best practises* and *challenges* within biomedical research facilities. Below follow three themes:

Protect research and personnel by accurate facility design and process work flow
- How can an automated cage reprocessing system within your facility layout, secure a cost efficient process flow and at the same time protect your research and personnel?

Steam sterilization – why preferable
- Why is Steam Sterilization a preferable method for achieving contamination control, and how do you overcome the challenges that occur with different loading scenarios?

Sterilization and Decontamination in bio-containment applications
- What critical requirements must be incorporated in the design of the sterilizer and its processes, while being used as barrier preventing biohazardous material from contaminating the BSL-3 or BSL-4 research environment or outside environment? Why cannot standard sterilizers fulfill these requirements?

OWE3

Better research through better reporting and reproducibility

Hasenau John[1], Honetschlager J.[2], Brayton C.[3], Kleinert M.[4] and Zynda J.[5]
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[5]*Academic Science Practice, Perkins + Will, Boston, United States*

Abstract
This workshop aims to consider practical options to achieve better research reporting and reproducibility. Participants will learn about reporting of environmental conditions for animal research models and next steps to ensure more rigorous reporting. The seminar will review historical concerns and contemporary issues going forward. Recent conferences have included much discussion on research reproducibility, and opportunities for improvements.

The workshop will:

1. Review developments and usage of reporting guidance
2. Review caging and equipment developments that aid in repeatability and rigor of the data reporting.

The workshop will underline how environmental factors are under recognized as contributors to experimental variation, and how this information is rarely shared or requested. The workshop will analyse how academia, pharma and CROs are using newer technologies to acquire the environmental data and individual cage data emphasizing repeatability and rigor.

The workshop will present examples of the practical application of digital information within a unit, and across a global organization, and its effects on study reproducibility. Husbandry impacts on reproducibility and data consistency. While ''meets Guide standards'' is the ''company line'', the details of daily care vary between institutions. Differences in cage components, housing density, cage changing frequency and practices are all recognized to impact animal development, physiology and behaviour. Suggestions to improve reproducibility of rodent research will be presented. A panel discussion based on audience questions will end the session. The target audience includes all individuals engaged in research involving animal models.

OWE4

Advances in operational and technical husbandry aspects in rodent gnotobiology

Hasenau John[1], Theriault B.[2], Huston S.[3], Harmelin A.[4] and Bom J.[5]
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[3]*International Microbiome Center, University of Calgary, Calgary, Canada*
[4]*Department of Veterinary Resources, The Weizmann Institute of Science, Rehovot, Israel*
[5]*Axenic/Gnotobiology Facility Manager at IGC, Instituto Gulbenkian de Ciência (IGC), Oeiras, Portugal*

Abstract
The workshop will present how emerging research investigating the role of the host microbiome in health and disease has provided revolutionary insight into the complexity of microbiome influence on host immunologic, metabolic and physiologic pathways, however microbiome research remains in its infancy. Interrogation of the complex intersection of host microbiome and system response has led to the revalidation of existing mouse models of human disease and to the development of novel animal models. The complexity of traditional specific pathogen free mouse models and the influence of the microbiome on disease modelling and reproducibility will be explained by opinion leaders on mouse microbiomes. Experiences in the progressive development of mouse models will be shared together with the description of the value and limitations of each of the models developed and utilized as well as the success in the translatability of a human transplant models. Challenges and opportunities in support of microbiome research will be discussed. The challenges of existing facility design and equipment requires consideration of associated biocontainment and biosecurity concerns when using (human) microbiota. The integration of flexible film and semi-rigid isolator housing with biocontainment and bio-exclusion ventilated rack technologies for the demand of high throughput studies will be discussed. Presenters will share their technical experiences in managing a gnotobiotic facility with the current technologies for germ free and gnotobiotic research. This workshop is insightful for all individuals at all levels in laboratory animal care, interested in microbiota and rodent modelling including recent advances in humanizing rodent microbiota.

OWE5

Opti Health Monitoring programs, enhancing the 3R and the 3E (efficiency, efficacy and expenses)

Foa Massimo[1], Bonaparte D.[2] and Montesano A.[3]
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Abstract
With recent technical developments in the field of rodent health monitoring, veterinarians and facility managers now have a vast range of possibilities within their choices between different approaches such as; sentinel screening, resident animals sampling, environmental diagnostics and different combinations of the above. An expanse/array of data is now available with regards to the sensitivity of each platform on different sample types. The results show that, unfortunately, there is not yet the perfect solution. Moreover, with multiple possibilities, there are now challenges when trying to balance science, costs and workload.

With the help of Aurelie Thomas (DVM, Sanger Institute, UK), chair of the session, Alessia Montesano (DVM, Leibniz Institute, Germany) and Dolores Bonaparte (DVM, Champalimaud Foundation, Portugal) we will look at the results of their independent studies analysing and comparing different approaches in terms of efficacy, efficiency and expenses. The presented data and the following open discussion could be helpful to everybody willing to design their own *Opti HM* program, the ideal health monitoring for their facility.

OWE6

Technical solutions in cross-contamination, biosafety & related operational management

Hardy Patrick
Veterinary and Professional Services, Allentown, Paris, France

Abstract
The design and operational / health management of laboratory animal facilities are submitted to growing and ever-increasing complexity, constraints and requirements, in order to respond to multiple objectives: quality and efficiency of the health management programme, overall performance of flow management (control of operations and cross-contamination risks), cost optimization (capital and operational expenditures), working conditions, implementation of ergonomics and OHS programme, contribution to sustainable development expectations... Responding to these challenges in a relevant and consistent way requires ensuring a technological watch, identifying, assessing and comparing all applicable solutions before deploying the most appropriate one, according to local specificities. The workshop aims at setting the scene of these challenges and sharing practical examples of effective solutions, responding to constraints such as implementing regulations and standards and taking into account operational efficiency and the nature of projects or studies conducted.

Example 1 – Selecting and integrating technical solutions in health and biosafety management: Criteria and comparative decision process in favour of one or several caging solutions, with regard to the animal facility design and flows, operational limitations, the nature of projects and studies, waste management and biosafety / OHS constraints. *P Hardy, Allentown*

Example 2 – Defining and assessing the performance specification of a rack / cage washer, defining suitable washing programmes and conducting the qualification phase. *O Nossan, Allentown-A.Co*

Exemple 3 – Indicators and techniques for performance qualification of washers and washer-disinfectors. *E Pastre, Theraxel*

OWE7

Development of hybrid health monitoring programs in laboratory rodent facilities

Fitzner Toft Martin and de Boer R.
QM Diagnostics, Nijmegen, Netherlands

Abstract
Traditionally, laboratory rodent health monitoring has been based on submission of live animals to the health monitoring laboratory and the application of microscopy, serology, PCR and culture to directly or indirectly identify specific pathogens in the animal facility.

In the past decade, application of improved molecular diagnostic methods has increased significantly, and the introduction of new sample types have enabled facilities to develop hybrid health monitoring programs making use of a variety of samples and testing methodologies to increase sensitivity and decrease cost, while improving animal welfare. Especially the introduction of environmental samples in combination with molecular diagnostics appear to be a valuable addition to most programs, but the nature of the sample and the superior sensitivity of the test method necessitates a new approach when results are interpreted, and potential sources of infections are identified.

This workshop will take a practical and participant-oriented approach to the evaluation of when and how new sample types such as on-site sampling kits and environmental samples from IVC exhaust air could be implemented and how it affects sample size and thereby the sensitivity of the overall program. Through practical examples, interpretation of results obtained from environmental samples and development of confirmatory sampling plans are discussed.

The objective of the workshop is to enable facility managers and laboratory animal veterinarians involved in designing health monitoring programs to make better use of multiple sample types and test methods to achieve superior sensitivity of the overall program while staying within budget.

OWE8

Sterilization systems for animal facilities

Bönisch M., Krage S. and **Schneider Andreas**
MMM Group, Planegg, Germany

Abstract
1. Special programs and procedures for the sterilization of feed and litter in animal facilities. Explanation of the different sterilizing processes and methods. Various methods for solid, porous and liquid goods. Required technical equipment in a steam sterilizer such as condensate sterilization or exhaust air filtration etc.
2. Maintain quality of reprocessing by re-evaluation and validation. Practical explanations of hygroscopic condensation – the lowest possible temperature effect on animal feed – steam and condensate removal from the goods.
3. H_2O_2 connection for combined sterilization of thermal instable goods. The concept and main requirements of a steam sterilizer with H_2O_2 connection used as transfer hatch. What relevant security systems need to be implemented?
4. MMM Group products for animal husbandry. MMM steam sterilizers, methods and options that fulfil the requirements of an animal facility.

OWE9

The real key to experiment success: Genetic stability over time

Leblanc Robert
JANVIER LABS, Saint Berthevin, France

Abstract
Two rodent statuses can interfere with experiment results: the Genetic Status and the Health Status. The Genetic Status is the most complex and the main issue with the Genetic Status is Genetic Stability of rodents over time, today as well as over decades to come, in order to ensure that researchers have constant, repeatable and stable experiment results.

At JANVIER LABS Genetic Stability is maintained by respecting the fundamental rules of genetics through:

1. Rigorous selection of breeders in order to create a stock of embryos. This selection has been carried out whilst conducting considerable genetic, phenotypic, haematological and biochemical controls.
2. Cryopreservation of a very large quantity of embryos of all our strains in order to guarantee users a Genetic Stability of all our strains for over 20 years.
3. Management of breeders in animal facilities: every 10 generations, we recover frozen embryos and replace ALL the breeders of our strains entirely in order to eliminate with certainty drifts or natural mutations which appear inevitably over time.

Guaranteed Genetic Stability is an essential pre-requisite for researchers for the continuity and repeatability of experiment results and JANVIER LABS conducts the Genetic Management for this.

OWE10

Maintaining a static microbiome across your research projects: Can it be done?

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Abstract
The microbiome of mice and rats have largely been ignored for general basic research until recently. In the past few years, specific microbiome studies have demonstrated that research results using animal models may have been influenced by an unchecked and unmonitored microbial consortia, and is impacted by virtually all investigated husbandry and housing variables. At the phylum level, barrier rooms which house standard strains in open-top cages may appear to be similar among a single vendor's barrier

rooms within strain, sex, and age. The reality is that at the genus and species level, there are usually differences in the diversity and abundance of bacteria that may provide research outcomes that are room, and possibly time specific. As researchers are starting to take note of which room they receive standard mouse strains from and question, ''what is the consortium within the rooms,'' vendors may consider providing routine monitoring of the microbial flora as part of the model characterisation. Can vendors be expected to maintain a near static microbiome status in the barrier rooms or other housing units? What can vendors and basic lab animal researchers learn from microbiome researchers who have brought forward the awareness of microbial drift? This workshop will focus on the challenges of maintaining a static microbiome among different housing schemes used for basic research and the methods and monitoring procedures that can be implemented to mitigate microbial drift from rodent model production through research investigations.

0WE12S1

Does relative humidity affect reproducibility of animal research?

Bayer Andersen Kirsten, Ekkelund Petersen K. and Hedegaard Andersen C.
SCANBUR Academy, SCANBUR A/S, Copenhagen, Denmark

Abstract
Relative humidity levels below 30-40% in the laboratory rodent facility may increase the risk of certain physiological conditions in mice and rats, including skin and eye conditions and a delayed puberty in female mice. Likewise, a relative humidity level above 60–70% have been shown to induce the first estrus earlier in mice, and may induce a favorable environment for unwanted pathogens.

Ongoing studies show interesting preliminary data on rodent welfare and physiology when relative humidity is locally, accurately controlled with an accuracy of ±3% within the EU regulatory guideline levels compared to when relative humidity is controlled centrally, and thus fluctuating with the variable weather conditions.

- In a UK facility when tightly controlling relative humidity the amount of water mice drank changed in response to changes in relative humidity.
- A current study in a UK mouse facility is looking at the effects of improved control of environmental conditions on breeding parameters in mice. The study is looking at controlled relative humidity of 55% compared to building controlled.
- In a test study in Austria, aggression in male mice dropped when relative humidity was controlled at 55%.
- Due to user anecdotes suggesting improved results a study will commence in the UK to investigate the effect of relative humidity controlled at 55% on embryo transfer in mice.

We will present our findings and invite participants to share their experiences on the impact of – or the challenges of obtaining – controlled relative humidity in the rodent facility.

0WE12S2

The effect of controlled relative humidity on water intake of C57BL/6J mice

Ekkelund Petersen K.[1], Towns R.[2], **Bayer Andersen Kirsten**[1] and Hedegaard Andersen C.[1]
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Abstract
Food and water intake of laboratory animals are parameters measured in various research fields. The housing environment can affect these parameters e.g., it has been shown that relative humidity (RH) may influence food intake in rats. In the current study, we investigated the effect of RH on water intake of mice.

An air handling unit capable of controlling RH at cage level with an accuracy of ±3% was used with IVC systems. The first month RH was set to 65%, the next month to 55% and the last month to 45%. Identical IVC systems connected to an air handling unit not controlling RH and thus, with RH fluctuating at room level, was used for comparison. Female and male C57BL/6J mice were used (N = 35), with 2–4 animals housed pr. cage. Water intake was measured on a weekly basis as an average pr. mouse pr. cage. The statistical analyses applied were repeated measurements ANOVA. Daily welfare assessments were performed. The study was carried out under license No. X7069FDD2 issued by the Home Office, UK.

The mice housed at RH of 65% drank significantly less during the one-month test period compared to mice housed at room-controlled RH fluctuating at 30–70%. For the mice housed at RH of 55% and 45% respectively, one of the weekly water intake measures were significantly lower than the measures of mice housed under room-controlled RH. To ensure reproducibility of studies measuring water intake and related parameters, it may be of importance to steadily control the RH.

PA1

European Quality In Preclinical Data (EQIPD) educational program on preclinical research and data integrity

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Abstract
Robust data from animal experiments are key drivers for decision making in the pharmaceutical industry and in basic research. Recent publications report shortcomings in the robustness and validity of data from animal studies, which often impact the transition from preclinical to clinical testing. European Quality in Preclinical data (EQIPD) is an IMI funded project with 29 partners

from academia and industry, aiming to make a lasting change in the rigour and robustness of pre-clinical animal research. Researchers should be more aware of the criteria and principles which increase preclinical research robustness and quality, to make a sustainable change in the way animal experiments are conducted and reported. Developing an educational program ensuring research-community wide expansion of knowledge on these topics is therefore part of our approach. Our main objective is to provide an online training program aimed at early career scientists and quality professionals, through which essential training on quality principles can be achieved. The scope of our training program covers i.a. scientific integrity, experimental design, validity, data handling and statistics, transparent reporting, systematic review of animal studies, the set-up of academia-industry collaborations and the implementation of quality management systems in discovery research environments. For each of these, existing open access online training materials are being identified and evaluated with a view to being collated into a complete training program. In addition, a yearly EQIPD summer school provides the opportunity for face-to-face training and networking, to achieve cross-fertilization and sharing of expertise. For more information, please visit https://quality-preclinical-data.eu/.

PA2

3R-Schooling for Methodological Approaches to Reduce Animal Tests

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[5]*Virtual Center for Replacement – Complementary Methods to Animal Testing, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany*

Abstract
The Directive 2010/63/EU firmly strengthens adoption of the 3R principle throughout all use of animals for scientific (and educational) purposes. The goal of the here presented German BMBF project is to support these 3R-research activities by establishing an open access platform that aims to provide training in methods to replace, to reduce, or to refine animal experiments. The platform is named 3R-SMART (**3r-smart.de)**, and tailored to the needs of students, PhDs, graduated scientists and technical staff of universities, public institutions, regulatory authorities, companies, and ethics committees.

From March 2019, the website ***www.3r-smart.de*** will be online and can be used both by the specialists in the registration area and by laymen in a public accessible area. Over the next 24 months, the content will be expanded to include contributions from all cooperation partners. All methods presented are accompanied by supplementary information. A blog with news and updates, as well as a forum for the exchange of information in the field of experts are continuously being expanded.

The 3R-SMART project will focus on methods which allow replacement of animal experiments. These methods will be demonstrated by training videos of alternative methods which are internationally accepted (e.g. validated methods according to OECD guidelines; e.g. OECD test no. 442C-E skin sensitisation, 471, AMES-Test, and GIVIMP (Good In Vitro Method Practices)). To warrant consistency of 3R-SMART, videos will be presented based on the different key topics biologics, chemicals, vaccines, cosmetics, disease models, food, medical devices, and pharmaceutics.

PA3

ADVANCE@MRC Harwell – A new Training Centre for Laboratory Animal Science & Genetics

Hough Tertius, **Gardiner M.** and Wells S.
Mary Lyon Centre, MRC Harwell Institute, Didcot, United Kingdom

Abstract
In recent years there has been much publicity surrounding the critical analysis of biomedical data. The drive towards developing both experimental protocols and reporting methods which support increased reproducibility has been embraced by funding bodies, scientific journals, learned societies and the animal welfare community seeking a reduction in animal numbers with a concurrent increase in the usefulness of the data generated from *in vivo* models. Improvements in reporting have followed the publication of the NC3Rs ARRIVE guidelines and many studies and discussions have revealed the probable sources of variability. There is very little formal training offered in UK to support the increase in both the knowledge and technical skills required to support reproducibility initiatives. The MRC Harwell Institute is at the international forefront of the study of mammalian models of disease. The institute recently secured funding to construct a new training centre, Advance @ MRC Harwell, with versatile laboratory, conferencing and IT training spaces, set to open in 2020. The training centre will make an important contribution to scientific training in the UK. The existing training programme is focussed on mouse genetics, experimental design & statistics, *in vivo* skills, ethics & welfare and laboratory & technical skills. In order to expand the range of courses to be offered through the training centre, The MRC Harwell have forged links with a growing network of training providers, including academic organisations, societies and commercial firms who are keen to utilise the facilities in the future.

PA4

An international master program for laboratory animal science as a contribution to the 3R's

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Abstract
With the implementation of the EU Directive 2010/63 the principles of the 3R's (Refinement, Reduction, Replacement) are for the first time incorporated into the animal welfare law and knowledge on 3R's and alternatives to animal experiments has to be part of the qualification of personnel planning, performing and evaluating animal experiments. In 2015 we started an executive FELASA accredited master program in Laboratory Animal Science (MLAS) for the qualification as a LAS Specialist (incl. function A, B and D). The MLAS is designed as an international, English-language part-time course incorporating a blended learning concept with e-learning modules, practical skills training and modular designed visualizations of knowledge integrated in the curriculum and in the learning platform. Modules address ethics and legislation in relation to the use of laboratory animals; biometry, statistics, experimental design and facility management; alternatives to animal experiments and laboratory animal science (incl. genetics, breeding, anatomy, physiology, pathology, hematology). For deeper knowledge courses in animal models, anesthesia and experimental surgery as well as in vivo pharmacology, applied toxicology, microsurgery and imaging are offered as compulsory and elective modules. In all modules 3R's are addressed and applied. Within the attendance block practical skills are taught by using stuffed animals, videos and training models like the silicon ear to shorten the learning curve when finally trained on animals. In addition, in vitro and ex vivo techniques are taught during practical skill courses. This should qualify persons responsible for directing animal experiments to use and advice other researchers in using alternative strategies.

PA5

Expanding the 3R concept into a guiding principle in veterinary training

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Abstract
Appropriate education of future veterinarians is not possible without real hands-on experience on living patients. However, professional implementation of practical training is hampered by the fact, that since the amendment of the Animal Welfare Act (2013) in Germany, teaching contents, when accompanied by exercises on living animals, are categorized as animal experiments. This results in new responsibilities in the usage of animals for educational purposes. In order to meet these plus the requirements of high levels of animal welfare, the Justus Liebig University Giessen (JLU) developed a novel step-by-step plan and thus extends the limits of the 3R concept to veterinary training:

First, students are taught the idea of animal protection through lectures and they are sensitized to a respectful handling of experimental animals. Secondly they practice basic veterinary and special clinical skills with various models (e.g. for injection, auscultation, intubation) at the Clinical Skills Lab "PETS- Practical Experience of Technical Skills ". In the next step students learn how the usage of Medical Training leads to the cooperation of the patient through targeted modification of behavior. By the final contact with living animals, students have routinely acquired the necessary competence through training, so that stress and strain for the animals can be reduced to a minimum and a significant decrease in the number of experimental animals (propaedeutic animals) is achieved. This project expands the 3R concept beyond animal experimentation to a sustainable training of future veterinarians who are not only experts in clinical issues but also in animal welfare.

PA6

The French veterinary specialisation in Laboratory Animal Science and Medicine: a status report

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[10]*COF_SMAL, Gidy, France*
[11]*LD Mirabel, Puylaurens, France*

Abstract
In 2010, the Directive 2010/63/EU introduced new requirements to protect animals used for scientific purposes. Establishments that

breed, supply or use laboratory animals are required to have a *''designated veterinarian with expertise in laboratory animal medicine, . . ., charged with advisory duties in relation to the well-being and treatment of the animals''* (Art. 25 of Directive; Poirier *et al*., 2015).

The French organisation of Veterinary studies includes two post-graduation Diplomas, the first level is CEAV (one year syllabus, ''Certificat d'Etudes Approfondies Vétérinaires'') and the second level is DESV (3 years, ''Diplôme d'Etudes Spécialisées Vétérinaires''), the latest enabling the Title of Specialist.

Those two levels have been created in Laboratory Animal Science and Medicine, covering the program of veterinary care according to the 2010/63/EU requirements (http://ec.europa.eu/environment/chemicals/lab_animals/pdf/Endorsed_E-T.pdf). The CEAV outcomes are based on a broad grasp in one animal facility, and the DESV outcomes add deeper requirements of professional or associative commitments. The DESV may be considered as the first step towards ECLAM Specialisation.

So far (period 2014-2018), a total of 53 veterinarians, from several countries, have submitted their credentials and have been admitted to the CEAV Diploma (21) and the DESV Diploma (32). We consider their achievements as a good way to promote the particular and advanced competences of Laboratory Animals Veterinarians. Beyond their diverse activities and competences, their commitment to a Diploma course recognized by peers but also by the National Authorities values a responsible use of animals for the advancement of sciences under conditions acceptable to the general public.

PA7

Undergraduate and taught postgraduate education in research animal sciences: A comprehensive learning outcomes framework

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Abstract

Despite advances in molecular and cellular research techniques, studies involving research animals remain essential, both in furthering our knowledge of animal biology and improving the health and wellbeing of humans and animals. It is critical that knowledge and understanding of the discipline is embedded within all Bioscience undergraduate and taught Masters programmes where students are expected to analyse and critique literature and/or data that has been generated from animal studies, irrespective of whether or not their Institution has research animal facilities.

The British Pharmacological Society, in partnership with experts in education, animal welfare and from Industry has created a research animal sciences learning outcomes framework: core learning outcomes for all relevant students (that doesn't require any hands-on animal work), and experiential learning outcomes for those students who wish to gain hands-on experience[1]. Prior to its launch, this framework was endorsed by 34 Organisations (Universities, Industry, Learned Societies, Animal Welfare organisations)

Going forward, to support educators in the delivery of this framework, individual learning outcomes have been mapped onto resources within ETRIS[2], a repository of free, online research animal sciences educational resources. The British Pharmacological Society is also working in partnership with the (UK) Physiological Society to facilitate the embedding of these learning outcomes into relevant degree programmes. Successful implementation will result in students graduating with the knowledge and understanding of the need to use animals for research where appropriate to address a specific scientific question, and of good practice in research animal sciences and welfare.

PA8

ETRIS: A free web-based repository of educational & training resources in laboratory animal sciences

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Abstract

There is a global shortage of individuals with the knowledge, skills and expertise necessary to undertake studies using laboratory animals. The use of animals for education and training is contentious, and therefore there is an increasing use of digital resources to supplement hands-on experience.

Whilst many excellent e-learning resources exist, most are unavailable to researchers, locked behind commercial or organisational websites. Those that are freely available are hard to find. To address this problem, ETRIS1 (Educational and Training Resources in In-vivo Sciences) was developed. ETRIS is a free website, which provides direct links to free, open access e-resources which deliver training or facilitate research involving animals; no registration is required. Individual resources are accompanied by a descriptive paragraph, which outlines what is in the resource, who developed it, copyright or access restrictions and suggested usage or audience. Resources are grouped into categories including animal welfare and husbandry, ethics, the 3Rs, experimental and statistical design, and surgical procedures. Additional categories will be added as the website expands.

The vision is for ETRIS to be a living repository, which grows as colleagues submit more resources for inclusion. If you have, or know of, any e-learning or training resources that you are willing to share please get in touch. We also want ETRIS to be adopted and, more importantly, used by laboratory animal personnel across the world. Please use ETRIS, link to it from your Institutional or company Biomedical Services or national Society websites, share it with your colleagues at home and abroad.

PA9

Australian veterinary and veterinary technical student views of animal use and impact of education

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Abstract
Currently in Australia there are limited opportunities for training in technical skills and comparative medicine of common laboratory animals. Specific topics including animal welfare, basic handling and procedures in commonly used laboratory species, or regulatory information are restricted in the veterinary or veterinary technical curricula at Australian Universities. When addressed these are generally covered as elective courses or minor components of a more general course. We hypothesized that this lack of specific training results in a shortage in qualified animal welfare officers and laboratory animal veterinarians available to the growing biomedical industry.

We hypothesised that the current veterinary science and technology curricula is inadequate to prepare students for careers in Laboratory Animal Science and Medicine (LASM). Further, there is a lack of awareness of, and preparation for LASM careers. A survey was conducted on veterinary and veterinary technical students at The University of Queensland to explore the level of acceptance, the awareness of LASM and the effect of education on these issues. This presentation will address our preliminary findings of a low level of support for animal use in biomedical research, lack of knowledge of LASM, lack of interest in LASM careers, as well as the lack of impact of the Australia curricula on engaging these students in this area. We will also introduce the future directions and international scope of this research.

PA10

The EUPRIM-Net NHP-specific FELASA course

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Abstract
To ensure that non-human primates (NHP) receive adequate care and handling in keeping and experiments, all personnel involved in the work with NHP have to receive appropriate education and training. Within EUPRIM-Net II (2011–2015) an NHP-specific Laboratory Animal Science (LAS) course was developed that takes place at the German Primate Center (DPZ) in Göttingen, Germany. This course, that is offered 2–3 times a year, consists of an e-learning period and a one-week on-site part and received full accreditation by the FELASA in 2018. The course is designed for persons carrying out procedures on animals (Function A) & designing procedures and projects (Function B). While the e-learning part covers important theoretical frameworks (e.g. legal aspects), the on-site part focuses on practical based contents (i.e. methods in NHP research). Since using NHP for education purposes is forbidden, participants learn amongst other practical contents behaviour monitoring, flushing of neuroscience head plants, principles of clicker training, and more. We are currently working on the creation of silicon models in order to offer more veterinarian practices in the near future. The course puts a particular focus on ethical and animal welfare aspects in order to sensitize participants for these issues, their responsibilities towards the animals in their care, and encourage critical thinking and evaluation of their own work at any stage along their careers as scientists in NHP research. Working with NHP in the laboratory demands particular education and training. Our course provides a solid foundation for personel working in NHP research.

PA11

A new approach for harmonising the Laboratory Animal Science education and training in Europe

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Abstract
The Directive 2010/63/EU on the protection of animals used for scientific purposes establishes that ''Member States should ensure through authorisation or by other means that staff are adequately educated, trained and competent''.

Since the minimum requirements regarding higher education or training for the acquisition, maintenance or improvement of vocational skills in Laboratory Animal Science (LAS E&T) are at discretion of the EU Member States (MSs), different regulatory scenarios are found on **LAS E&T** in Europe. This fragmentation hinds mutual recognition of training and free movement of professionals in the EU.

In the framework of the Erasmus+ Programme, the so-called ''**HERMES project**'' (www.hermes4las.eu), promoted by a European consortium gathering stakeholders from Belgium. Italy, Greece, Spain, and Sweden, addresses the challenge to support MSs in the mutual recognition of LAS E&T. The solution is a new **voluntary Quality Standard** for LAS E&T providers. It allows the management of setting-up, deliver, monitoring and evaluation of training compliant with the Directive 2010/63/EU.

The design of the Hermes Project Quality Standard for LAS E&T arises from the consensus achieved by EU stakeholders' representatives through different knowledge elicitation techniques (Delphi sessions and focus groups). This intellectual output represents the cornerstone of a structured model that starts from the self-assessment of the LAS E&T providers (with respect to the standard), and culminate in a multi-language European Digital Platform to certify – automatically and on a common scheme – the skills of the personnel foreseen by the Directive 2010/63/EU.

PA12

IACLAM Training Taskforce: Quality assessment of formal training requirements for colleges of LAM globally

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Abstract
The International Association of Colleges of Laboratory Animal Medicine (IACLAM) was formed in 2005 from a unique partnership between the European, Japanese, Korean, and American Colleges of Laboratory Animal Medicine. One important objective of IACLAM is to support high quality education, training, and continuing professional development of laboratory animal veterinarians around the world. As part of this, a recent project for IACLAM has been to review training program requirements for the four colleges with a long term goal of harmonizing global expectations for training and education of laboratory animal veterinarians, and, in particular, those that are seeking to become specialized in the field. To better understand the requirements of each college in this regard, IACLAM conducted a detailed review of college-specific training and quality oversight processes for residency and post-graduate training programs in laboratory animal medicine. Three of four colleges have formal approved training programs (i.e., residency or graduate student programs) with lesser numbers of recognized training programs in newer LAM Colleges. While KCLAM does not have formal standalone residency programs they do have a proscribed duration of mentoring, formal coursework, and publication requirements for veterinarians to complete prior to becoming eligible to sit the certification exam. While all colleges specify a required number of hours of didactic training for formal trainees, ACLAM specifies the minimum duration of applied training over the course of the training program. This process has permitted IACLAM to more carefully evaluate training programs between colleges and these and other findings will be further highlighted.

PA13

Treating students as new Principal Investigators: A novel method for animal research facility outreach

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Abstract
The University of Illinois' outreach program began when a research advocacy organization contacted us on behalf of a professor at an unaffiliated university. The professor taught a class in animal philosophy and sought an opportunity for students to visit an animal research facility. University veterinarians, the research advocacy director, and the professor spoke by phone about the guidelines and expectations for the animal facility tour. All parties agreed to not only tour students through the animal facility but to treat them as new principal investigators (PIs). Prior to the visit, students completed Occupational Health and Safety (OHS) and Institutional Animal Care and Use Committee (IACUC) online training and reviewed a rodent research protocol. On the day of the tour, veterinarians and IACUC staff presented lectures on animal care, regulations, and protocol review to the students. A veterinary technician presented a training module about rodent experimental procedures. An animal facility supervisor toured the students through the animal facility and presented the same orientation material that new PIs receive, including how to properly restrain a mouse and change a cage. Afterwards, a campus researcher presented information on oncology studies that benefitted humans and animals. Throughout the day, there was time for questions and answers, which contributed to meaningful dialog. By treating students as new PIs, we were able to actively engage them in the orientation process and to demystify the use of animals in research.

PA14

Raising the bar: Africans skilled in humane animal care and use for scientific purposes

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Abstract
Animal use in research in many African universities and institutions lacks appropriate operational quality assurance. Many African universities and institutions with laboratory animal holdings, today use animals in experiments for biomedical and other kinds of research without the benefits of any form of regulatory guidelines, either legislative or non-legislative, although many African countries are signatories to OIE – World Organization for Animal Health charter. There is, therefore, a requirement for an adaptable set of operational core values and guidelines. ''ACURET-Raising the Bar'' project provides innovative strategies to increase the number of skilled researchers and technicians in humane animal care and use for scientific purposes in Africa. The overarching goal of these strategies is to make every animal life count where and when it must be taken. This project is the development of a data-driven strategic self-voluntary regulatory mechanism cognizant of cultural, socioeconomic, political and religious diversities across the African continent. Beginning in 2017, ACURET evolved a Directorate of Forum, Training and Education (FTE), whose activities resulted in the training of 65 animal users in Nigeria, Ghana and South Africa, with possible extension to Ethiopia in 2019. It is envisaged that in a sustained manner, and with encouragement and support, that this continent would seamlessly blend with the rest of the world in matters of laboratory animal care and welfare.

PA15

Laboratory animal sciences course in Algeria: An initiative to implement animal welfare and education

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Abstract
A mandatory training in laboratory animal science in Algeria implemented since 2013. However, a comprehensive evaluation needs more analysis and consideration following significant demand for training and education on animal welfare from scientists and personal using animals for scientific purposes. The purpose of this work is to analyze current trends in change on the importance of animal welfare education in Algeria and the competencies recommended by international education organizations for the region. In 2018, the Algerian Association of Experimental Animal Sciences (AASEA) in collaboration with the International Council of Laboratory Animal Science (ICLAS) and the *Federation of European Laboratory Animal Science Associations* (FELASA) and in partnership with the National High School of Veterinary and Pasteur Institute of Algiers organized the first international course of animal experimentation. The participants were veterinarian, scientists and post-graduate students from different institutions. The course evaluation survey showed that the whole topics and elements, especially theoretical knowledge and practical skills on animal handling and procedures using live anaesthetized animals, cell culture and immunization methods, were widely appreciated and a large majority of participants considered that the 3Rs principles, were correctly applied during the course. Despite modest progress in introducing animal welfare education and training programs for scientists and veterinarian remains to be done regarding the future in this field in teaching laboratory animal science in Algeria. Finally, we think that is necessary including this science with collaborative learning as integral course part in educational programs to integrate the scientific, ethical and legal aspects of animal welfare.

PA16

Development of an effective 3R teaching strategy and practical 3Rs education and training resources

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Abstract
In order to further promote the implementation of the Directive 2010/63/EU, the European Commission and the Joint Research Center (JRC) issued calls for a number of related projects. One of these projects is currently being executed by ECORYS and SYRCLE in collaboration with the JRC. The aim of the current study is to provide input for a number of JRC activities related to the dissemination and promotion of alternative methods and the Three Rs. The first part of this study should identify key elements that should be considered for the development of guidance for educators for incorporating Three Rs into their curricula. The second part of the study will specify and develop practical Three Rs education and training resources. For the education and training resources three specific groups are targeted, namely, high school students, university undergraduates and early career scientists. Experts from a wide range of backgrounds, e.g. policy makers, 3R experts, curriculum experts, and representatives of European school and university organisations are being interviewed. A summary of these interviews will subsequently be discussed in a stakeholder meeting with selected experts. Also a rapid evidence assessment of already existing educational materials in the field of the 3Rs will be taken into account. The results of these interviews, the rapid evidence assessment and stakeholder meeting will be presented, as well as the draft plans for the second part of the study. Also it will be explained how this project links to the other projects initiated by the European Commission and JRC.

PA17

Experiences with the reorganization of classical FELASA courses into e-learning and practical training modules

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Abstract
The requirements on education and training of personnel involved in use of animals for scientific purposes are defined by the EU-Directive 2010/63 and the corresponding working group document[1]. In order to implement this new framework, existing laboratory animal courses have to be transformed into a modular concept consisting of theoretical core and function specific modules and practical task specific training. There are several options

to meet these requirements. In our institution we decided to perform theoretical parts by means of e-learning and associated success control, which serves as admission requirement for task specific practical modules. Restructuring of temporary lectures on e-learning has the main advantage that learning contents are always accessible to scientists in a flexible way. Practical modules are offered almost monthly and can be completed individually, which also contributes to this flexibility. Depending on required qualification for the respective animal research project, participants will be educated and trained in appropriate skills (i.e. only participants who will carry out surgical interventions get training in surgery). The modular concept allows scientists to complete targeted training in a timely manner and according to their demand. Finally fewer animals are needed than in classical non-modular FELASA courses. Furthermore, all modules build on each other and are offered throughout the EU according to the same concept. That enables to extend the individual qualification if necessary at a later date or in a different location. In conclusion this modular training framework offers clear improvements in terms of better flexibility, training outcome and animal welfare.

PA18

The impact of trainee characteristics on practical training scores in LAS courses

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Abstract

Background: Education and training in laboratory animal science is a prerequisite for persons desiring to perform the four functions, according to the Directive 2010/63/EU. Our aim was to investigate differences or correlations between trainee age, gender, degree, previous practical experience and the practical competence acquired as evaluated by their scores in mice and rat handling, restraining and minor procedure performance.

Materials and methods: In a 4-year period, 78 trainees participated in the LAS European Union Functions Course held in Athens, Greece. The trainees were trained by 1 trainer and evaluated by 2 independent evaluators in handling, restraining and performance of minor procedures. A correlation between trainee age and practical score was evaluated, while comparison of their practical scores was performed according to their gender, degree and previous experience.

Results: No correlation was evident between age and scores in mice ($p = 0.129$) and rats ($p = 0.884$) as evaluated by Pearson correlation coefficient. Gender had no impact on practicals' score ($p = 0.312$) regarding mice, while a significant impact was observed regarding rats ($p = 0.042$). No difference was documented between 5 groups having different degrees (veterinarians, doctors/dentists/pharmacologists, technicians/nurses, biologists, physical scientists) in mice ($p = 0.803$) and rats ($p = 0.706$) and no impact of previous experience was evident on scores for mice (0.341) and rats (0.610).

Conclusions: According to our evaluation no impact of age, degree and previous experience was observed for practical scores on mice and rats. Impressively, gender had an impact on rat practical scores.

PA19

Mouse dummy for intravenous application into the lateral tail veins

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Abstract

Background: Every year, many mice are used in Germany for training and (further) education. Training on living animals is necessary and not entirely irreplaceable in order to teach and learn the required handling skills. But dummies play an increasingly important role; they can be used to train skills before using live animals. The dummies currently available on the market already offer a variety of training options (e.g. per oral, subcutaneous, intraperitoneal and intravenous applications), but they are very expensive and there are only a few professional dummies available on the market (as for mice, there is only the Mimicky Mouse©).

Methods: 3D printed mouse with silicone tail including two catheters simulating lateral tail veins. ELISA-Measurements of corticosterone levels in serum of mice. Survey for participants to evaluate their training comfort.

Results: In our ongoing study, the study participants (grouped in animal caretaker trainees, fully trained animal caretakers and scientists) first performed the intravenous application in mice without previous training on the dummy, in the second part of the study the participants have trained with the dummy before and then they performed the intravenous application on mice again. We measured corticosterone levels in serum of all treated mice basal, with and without previous training on the dummy. First results show significantly reduced corticosterone levels in mice where participants had previously practiced with the dummy.

Conclusion: Our ongoing study shows that the use of our mouse dummy results in significantly reduced stress levels in mice compared to training without a dummy. Our survey also shows that the learning comfort of the participants is increased.

PA20

Introduction of a silicone embryo model for teaching in the biomedical sciences

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Abstract
In 2016, about 2.8 mio. animals were used in biomedical research in Germany, the number of mice used for research being 2 mio. The principles of 3R – reduce, refine, replace – give us an ethical guideline for experimenting with animals, with the replacement of animals being the final goal.

With 3D-printing technology coming up in the last years, we see new possibilities to replace animals in research and teaching. One instance being the teaching of surgical techniques such as the *In-Utero*-Elektroporation (IUE), a technique which is commonly used in Neurosciences that allows genetic modification of the developing brain of a mouse embryo inside the uterus by intraventricular injection of a vector and applying an electrical pulse .So far, each trainee uses 25 mice, until this complex procedure can be performed independently.

By using 3D-printing technology, we created a realistic embryo silicone model, on which new trainees will be able to practice the crucial steps of the IUE, such as the injection in the ventricle and the proper placement of the electrodes, without using a live mouse.

This possibility will heavily reduce the number of mice used for learning the IUE and as it can be easily extended to other organ systems it can effectively replace mice used in teaching for biomedical sciences and research, as Russell and Burch outlined in their work on the 3R-principles.

PA21

Acquiring confidence in handling and restraining skills in rodent courses

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Abstract
Background: Laboratory animal science courses that train Function A, C and D personnel according to the Directive 2010/63/EU require practical competence of trainees in handling and restraining (H+R), namely Module 3.2. Most rodent courses introduce the procedures with slides or video presentations, before proceeding to practical sessions with live mice and rats.
Materials and methods: The current course has been running for three years with the above-mentioned educational sequence. In an attempt to promote the application of alternatives, to increase trainee confidence and reduce animal stress, following the respective species H+R powerpoint presentations, each trainee was given a mouse- and a rat-sized and -shaped dummy. They were individually trained on these, mastering the different finger positions and techniques before proceeding to H+R live mice and rats.
Results: The trainees, depending on their scientific background, needed more or less time to master the different H+R techniques on the dummies. When proceeding to the live animals, they demonstrated evident calmness and confidence in their movements of a greater degree and swiftness than trainees of previous courses that lacked this step. Vocalization and escape efforts of the animals were minimal. All trainers confirmed and were pleased with these observations.
Conclusions: The use of dummies as alternatives prior to training H+R techniques on live rodents is beneficial to the trainees' sense of confidence and their acquiring competence. It significantly contributes to the animals' welfare as they sense this confidence and are minimally stressed.

PA22

Transition to alternatives to lab animal use in veterinary education

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Abstract
In 2016, the Netherlands National Committee for the protection of animals used for scientific purposes (NCad) formulated a number of strategic recommendations to accelerate the national transition process towards animal-free innovations in research and education. The Faculty of Veterinary Medicine of Utrecht University proactively endorses the NCad point of view of an 'animal-free mindset' within education and training.

The use of animals and alternatives in veterinary education at Utrecht University in relation to the learning goals has been investigated by a project team consisting of representatives of the faculty of Veterinary Medicine, students and coordinators of animal related education, the animal welfare body and critical outsiders. Subsequently, an analysis of the options for replacement, reduction and refinement of animal experiments in veterinary education was performed. Based on this analysis, concrete proposals with immediate impact, as well as strategic advice have been put forward that replace, reduce and refine the use of animals at all levels of veterinary education while keeping in mind the constant need for trained and skilled veterinarians now and in the future. Examples include the purchase of futuristic animal models such as the SynDaver Surgical Canine and overarching advice on implementation of a Code of Conduct towards animals in education.

PA23

Rabbit anaesthesia – Developing animal technician competence and confidence

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Abstract
Rabbits have a reputation for having one of the highest published anaesthetic death rates of any companion animal species.

For animal technicians without an extensive background knowledge in anaesthesia, facing this procedure can become a stressful and daunting experience. This stress can also contribute to an undesirable outcome.

The presentation will describe the reasons why rabbit mortality is higher under anaesthesia comparing with dogs and cats, and to review the essential critical points unique to rabbits. This then allows an anaesthetic plan to be made using the appropriate tools and techniques to overcome these challenges.

These tool and techniques will provide animal technicians with the necessary confidence to perform this procedure safely and calmly, leading to a successful outcome and refinement for animal welfare.

PA24

Skills achieved to carry out procedures on mice and rats after initial practical training

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Abstract
The time taken to achieve the EU learning outcomes on practical skills for procedures [EU 3.2, 6.2 and 8] on animals is known to considerably vary among trainees. The aim of this study was to describe the clinical competence achieved by undergraduate students within 9-hour practical training of minor procedures in both mice and rats for quality assurance. Prior to starting the hands-on sessions using animals, a survey was conducted to ask about each student's (n=62) previous exposure to handling mice and rats, and about their experience with minor procedures. According to trainee's own perceptions, 82.26% did not have any or little previous experience with handling mice and rats, and 85.48% did not have it with minor procedures. At the end of the hands-on sessions, assessment of skills was individually evaluated by the assessor using Direct Observation of Practical Skills and the Miller's classification for clinical competence. Results for mice revealed that 42% of trainees were able to handle and restrain them at least once, 55% were able to show how to carry out minor procedures, and 60% learnt how to kill a mouse. Findings for rats revealed that 44% of trainees were able to handle and restrain them a few times, 61% were able to show how to carry out minor procedures, and 53% learnt how to kill a rat. Although none of the students was able to become competent to work without supervision, we conclude that students were able to gain relevant practical skills.

PB1

Laboratory animals in the research of airway defensive reflexes

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Abstract
The most important information about neural pathways in cough and its pharmacological regulation have been conducted in guinea-pigs, rabbits, cats and dogs. From small laboratory animals, the best model is awake guinea pig. Neurophysiology and neuropharmacology of the vagus nerve in guinea pigs is very similar to humans and opens possibilities for successful translational research. Although many studies have been performed in conscious rats and mice, there is scepticism regarding the ability of these animals to cough that resembles the reflex seen in human due to the lack of particular airway afferents. Conscious animals are placed into a plethysmograph box where they inhale aerosol of tussive agents and the count of coughs is recorded. Up-regulation or down-regulation of cough can be modelled by different approaches, e.g. sensitization of animals, exposure to the tobacco smoke or medication with ACE-i. Nowadays, possibility of the use of SPF guinea pigs is being discussed, because their cough response is weak. Large laboratory animals such as cat or rabbit are used as anaesthetized tracheotomised model. In these models, cough is induced either by inhalation of tussive substances of by a mechanical stimulation of the airways. Cough is recognized based on a large burst of electromyogram activity in the diaphragm immediately followed by a burst of activity in the rectus abdominus muscle. Interpretation of the results must consider the differences conserved across species and the results obtained in laboratory studies must be translated to the clinical conditions with understanding of all limitation of used models.

PB2

Experimental design on slow progressing murine tuberculosis model to evaluate vaccine and treatment efficacy

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Abstract
Although the spectra of human and murine tuberculosis do not completely overlap, *in vivo* mouse *Mycobacterium tuberculosis (Mtb)* infection models provide many advantages including the susceptibility to infection, pathogenesis, immunology and controlled progression of the disease. Protocols utilized for TB research are differ in the route of infection, inoculum and strain of the bacteria, strain of mice, timing of vaccination and/or treatment, type of endpoint analysis, etc.

In our study intraperitoneal *(ip)* infectious route was chosen because, this way of infection induces a course of slowly progressive and systemic disease. Well-being of the animals, monitored by the body weight, allows a prolonged experimental set up and provides a great opportunity to test the long-term activity of antituberculotics and vaccines

Nine groups of 6–8 weeks old female BALB/c mice were used to test nanoparticulated *in silico* identified novel compounds and peptides-based vaccine candidates. The immunization was carried out three times (two weeks apart) and after incubation period mice were infected with *Mtb* H_{37}Rv. All work with infectious *Mtb* was performed under biosafety level 3 conditions. Non-immunized mice were treated with drug candidates twice a week, for seven weeks. After euthanasia, organ homogenates were cultured on Löwenstein-Jensen media and colony forming units (CFU) were counted. In histological sections rod-shaped acid-fast bacteria were observed in the organs of untreated control animals that indicates a successful experimental design to model tuberculosis. *Per os* administered nanoparticulated drug compounds showed relevant antitubercular activity and multiepitope-based vaccine candidates provided protective immunity against *Mtb* infection.

PB3

Establishment of a model of atopic dermatitis induced in dogs

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Abstract
Atopic dermatitis is a chronic relapsing and pruritic inflammatory skin disease, found in humans and dogs. The two main mechanisms involved in pathogenesis are structural and functional abnormalities of the epidermal barrier, on the one hand, and cutaneous inflammation induced by an inappropriate immune response to irritants and allergens entering the skin, on the other hand. Our goal was to establish a canine model of atopic dermatitis and to determine the most relevant clinical parameters to detect cutaneous inflammation during the early stages of the disease. Six healthy adult male Beagle dogs were used. The model of contact dermatitis was put in place for six weeks via the use of *Dermatophagoides farinae*. The cutaneous reaction was clinically, histopathologically and biologically monitored. With our protocol, all dogs, except one, developed skin inflammation. Skin fold measurement detected skin inflammation around the sixth allergen application. The induced dermatitis presented the main characteristics, histological and immunological, of spontaneous atopic dermatitis. Thus, our atopic dermatitis model, induced by *Dermatophagoides farinae*, is relevant and mimic the spontaneous disease. Skin fold measurement is the most relevant and simplest parameter to follow the early phase of inflammation. Additional studies on a larger number of animals are needed to validate our protocol and test different concentrations of *Dermatophagoides farinae*. This experimental model seems adapted to the study of T lymphocyte populations, their kinetics and their role in the initiation of cutaneous inflammation. In addition, this model can be used to test treatments for atopic dermatitis in dogs and humans.

PB4

Validation of the healing model in diabetic rat

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Abstract
Today with 425 million people suffering from diabetes worldwide, this pathology is considered as a major public health problem. With a prevalence at 90% that tends to increase, type II diabetes associate at the insufficient secretion of insulin, the insensitivity of cells to its action. Among the known complications, delayed healing is the major problem related to this pathology. In order to understand and study the phenomena involved in this process, it is essential to develop appropriate scar models. The objective of this study is to validate a scar model in rats and to characterize it by clinical scoring and planimetric analyzes. This validation is based on the comparison of the healing kinetics between two strains: ZDF diabetic's rats and WISTAR healthy rats. The data obtained did not highlight a scar defect into the ZDF rats. Indeed, none significant difference could be demonstrated between pathological and healthy rats. Due to the phenomenon of cutaneous contraction present in rats, results assume that this model is not adapted for research based on healing process. Presumably, pork would be a more appropriate and predictive model for studying these mechanisms.

PB5

The use of mini-pigs as a model for studying the biocompatibility of intravascular stents

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Abstract
Laboratory mini-pigs are considered the optimal object for experimental surgical operations. The possibility of using the pig as an adequate model of the object in biomedical experiments is predetermined by its resemblance to a human for a whole set of anatomical and physiological characteristics and biological properties. The similarity observed in morphology and function of internal organs and systems and in organization of metabolic processes.

In this paper, we study the structure and properties of self-expanding titanium nickelide stents subjected to plasma-immersion ionic modification with silicon, and evaluated biocompatibility of the stents at the implantation into the organism of experimental animals (miniature Siberian pigs – minisibs).

2 groups of animals, 3 mini-pigs in each, were investigated: the experimental group in the common carotid artery implanted with an experimental sample of titanium nickelide-modified stent, control group – commercially available sample without analogue processing. Endovascular implantation was performed in a minimally invasive manner using vascular access through the femoral artery under fluoroscopic control. Three months after surgery, control arteriography and ultrasound were performed to evaluate hemodynamics in the area of implantation. Were also performed in vivo studies of the interaction of experimental samples of stents with tissues and body fluids, including studies of the hemostatic system, coagulation, cytological, biochemical blood tests, pathological and histological studies. According to the results, it was shown that the investigated stents possess corrosion resistance and the absence of toxicity in biological media, and also do not have a pathological effect on the body of an experimental animal.

PB6

Surgical model of spinal cord injury in rats

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Abstract
The review of international experience based on the use of experimental models of spinal cord injury (SCI) demonstrates that there are need requirements for the standardization and validation of the injury inducing methods [Minakov et al., 2018].

This study was carried out in the Animal Breeding Facility of the Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences which has been fully accredited by the AAALACi. The model of minimal invasive structural SCI was developed on the SPF-category Wistar and SD rats.

Design of the experiment was developed including the following stages: modeling of a standard scar; a methods to remove the scar tissues; and ways to create conditions for axon regeneration. Researchers have selected an object for modeling that best matched the specific requirements of implantation techniques, identified and verified the location of experimental effects. Approaches to the preoperative preparation and post-surgery monitoring; anesthesia care; surgical access and operative wound closure were established in compliance with the 3Rs. Histological techniques are proven to verify the results of assessment of the experimental effects. Special conditions were selected to reproduce structural defects in the spinal cord of rats with similar morphometric characteristics. Some preliminary results of the spinal cord hemisection were obtained with the use of laser equipment and an original puncture technique, for taking sample from the frozen SC. The findings demonstrate that the developed SCI model was promising and may be appropriate for further experimental studies of the SCI with laser and cryo-technologies.

PB7

Utility of aging farm mice to use for geriatrics in NCGG

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Abstract
Our facility has kept many naturally-aged animals (mice and rats) used for gerontology and geriatric researches. If a scientist make a research using these animals which have been kept for long time, it is very important to consider the effects of various genetic and environmental factors on them. However a clear criterion for defining of aged mice has not been established. In the present study, we will report various age-related characteristics observed in our mice. Four weeks male and female mice (C57BL/6NCrSlc) were purchased every three months and kept over their lifetime. Physiological (measurement of body weight and survival rates), behavioral (the rotarod tests and social interaction tests) and morphological (autopsy, histological examination) analyses and hematological (WBC, RBC) analyses were performed. Body weight peaks at 18-20 months-old (M) (approximately male 46.0g, female 35.0g). Survival rates start to decline approximately 21 M, and are somewhat lower in female aged mice. Rotarod performance peaks at 3 M in male (208±61.4 sec) and at 6 M in female (235.3±75.1 sec) mice and then continues to decline. Blood test show that the total WBC count starts to decline at around 18 M tends to be change with aging. Various age-related changes (such as body weight, motor performance, and the total WBC count) found in our aged B6N mice can be candidate senescence indicators at individual level. We will add C57BL/6J mice, investigate these parameters in detail and continue to search for novel biomarkers (such as fecal IgA levels).

PB8

Can crickets replace mice in the venom toxicity test?

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Abstract
The assay of lethal toxicity in mice is an important step to assess (and compare) toxic activity of venoms. This assay causes animal suffering, pain and death, and employs a large number of mice. Several snake species consume arthropods, rather than mammals, so the toxicity test in mice might not be suitable for the evaluation venoms' toxicity.

There are three species of venomous snakes in Croatia: long-nosed viper (*Vipera ammodytes; Vaa*), European adder (*Vipera berus; Vbb*) and meadow viper (*Vipera ursinii spp; VuCro*). The first two eat mammals and birds, but *VuCro* vipers also include insects in their diet.

Here we investigate for the first time the composition and biological activity of the *VuCro* venom. The *VuCro* venom is less lethally toxic in mice than the *Vaa* venom. However, the pattern of mice dying indicates a presence of a strong neurotoxic component. Interestingly, two-dimensional gel electrophoresis revealed a lack of ammodytoxins, which are known neurotoxic components of *Vipera* venoms. Western blot of non-reduced *VuCro* venom with anti-ammodytoxin antibodies gave no signal. Mass spectrometric identification of SDS-PAGE bands showed the lack of ammodytoxins as well. This was finally proven by ELISA, in which *Vum* venom coated wells were not recognized by anti-Atx antibodies.

Since *VuCro* viper is an insect consuming animal, we investigated whether crickets (*Gryllus assimilis*) could be a better model than mice. Venoms' components with potentially insecticidal activity can't be assessed with a venom toxicity assay in mice because it would not be detected or might be missed.

PB9

Mouse adenovirus-1 and pneumonia virus of mice infection experiments in C57BL/6 and BALB/c mice

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Abstract
To secure reliable results from laboratory animal experiments, the use of specific and sensitive assays to monitor viral pathogens are obligatory. Health monitoring is currently based on molecular and serological investigations. To generate defined sera for improvement of serological diagnostic assays, low dose infections with MAD1 and PVM were performed.

MAD1 and PVM were cultured according to the American Type Culture Collection. Virus stocks were titrated to verify the amount of infectious virus in plaque forming units (PFU). Eight weeks old C57BL/6 and BALB/c mice were inoculated intranasally with MAD1 or PVM using 50 or 100 PFU. Serological investigations were monitored at days 0, 14, 28, 42 p.i. Clinical scoring was performed daily.

The immune response to MAD1 differed depending on the mouse strain used. MAD1 specific antibodies were detected in C57BL/6 mice two weeks p.i., whereas BALB/c mice remained antibody negative during this time period. Both mouse strains did not develope clinical signs in contrast to PVM infected mice, which showed notable weight loss one week p.i..

Low dose exposure of laboratory animals is often formidable, as subclinical signs and delayed immune responses hamper diagnostic efforts. Low dose MAD1 and PVM infections in C57BL/6 and BALB/c mice resulted in partially delayed immune responses and different clinical signs depending on the mouse strain used. Further investigations including the establishment of new immunofluorescence tests and/or ELISAs will be necessary to verify and proof the infection status in clinically healthy low dose exposed laboratory animals.

PB10

Characterization of Apolipoprotein E knock out mice aged as model of neurodegenerative diseases

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Abstract
Neurodegenerative diseases affect millions of people worldwide, where Parkinson and Alzheimer's disease are the most common types. The risk of being affected by a neurodegenerative disease increases dramatically with age. A knockout mouse model, B6.129P2-Apoe^{tm1Unc} is homozygotic for the Apolipoprotein E (APOE) deletion; thus, it is capable of developing hyperlipidemia and atherosclerosis but APOE is also a lipid-transport protein abundantly expressed in most neurons in the central nervous system. For this reason, the present work has as objective to determine the physiological and pathological parameters of the aged B6.129P2-Apoe^{tm1Unc} mice, as neurodegenerative disease model. For its development, they were carried out genetic controls by means of the Polymerase Chain Reaction technique, the determination of the growth curve and the parameters of hematology, sanguine biochemistry, organ weight and spontaneous pathologies. Besides, behavioral and learning tests were conducted as well as anatomopathological studies of mice's brain. As result, age dependent increases in cognitive impairment were detected in mice with ApoE deficiency although no changes in other hematological and biochemical parameters were founded in comparison with young animals; histopathological study evidenced enhance of lipid accumulation in the brains of aged mice. According to results, this model is potentially relevant to Alzheimer's disease. Thus, the apoE knock out mouse model should help us to understanding the underlying biological mechanisms of neurodegenerative disease and the role of cholesterol metabolism incognition. In conclusions our current data may help to provide a suitable animal model with potential application in neurobiological sciences

PB11

Suitability of AAV2/DJ-mediated alpha-synuclein overexpression as a rat model of Parkinson's disease

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Abstract
Neuropathological hallmarks of Parkinson's disease (PD) include progressive degeneration of dopamine (DA) neurons in the ventral midbrain and alpha-synucleinopathy. To establish a suitable PD animal model in rats, we mimicked the disease features by overexpression of either the human alpha-synuclein wildtype (WT) or the E46K mutant form (E46K) in DA neurons of the substantia nigra in rats using AAV2/DJ as viral vector. Transduction efficiency was compared to an equal virus titer expressing GFP. Motor skills of all animals were evaluated in behavioural tests every four weeks over a total time period of 12 weeks. Also, quantification of DA cells and measurements of residual striatal DA fibers were performed. The WT group showed a progressive loss of DA neurons with 40% reduction after 12 weeks, which was accompanied by a greater loss of striatal DA innervation. In contrast, E46K led to the same reduction already after four weeks, which did, however, not progress further. Insoluble alpha-synuclein positive cytoplasmic inclusions were observed in both groups within DA neurons of the ventral midbrain. In addition, both groups developed a characteristic worsening of the rotational behavior over time. Interestingly, only the WT group reached statistically significant different values in the cylinder test. Summarizing these effects, we established a motor symptom animal model of PD by using AAV2/DJ in the brain for the first time. Overexpression of alpha-synuclein E46K mimicked a rather pre-symptomatic stage of the disease, while alpha-synuclein WT overexpression imitated an early symptomatic stage of PD.

PB12

Metabolomics as a tool to improve phenotyping: Experiences with the biomedical pig

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Abstract
Animal models are constantly been set up, and pivotal step in such process is an in-depth genetic and phenotypic characterization. When it comes to qualitative/quantitative profiling of biological fluids, metabolomics-based approaches, in particular ^{1}H NMR spectroscopy, have been proved to be highly reliable in different species. Hereby, we report the used of the above-mentioned technique to characterize Cerebrospinal fluid (CSF) and Vitreous Humor (VH) of biomedical pigs. For the first study we investigated the composition of Cerebrospinal Fluid (CSF) harvested form healthy newborn (5 days old) and young (30 and 50 days old) piglets and evaluated any difference between age groups related to Blood-Brain-Barrier maturation[1]. On each sample, 30 molecules were observed above their limit of quantification. Only 11 molecules showed significant differences between P5 and P50 animals, giving some insights to the ever changing selectivity of the barrier. In second study, we investigated the composition of the VH in the standard pig and in a photoreceptor degeneration model induced with Iodoacetic acid (IAA)[2]. VH samples were collected upon vitrectomy and analysed by means of ^{1}H NMR spectroscopy. 40 molecules were identified and quantified, with lactate being the most abundant in both conditions. Upon comparison, 17 molecules showed statistical differences between groups and could potentially be further investigated as photoreceptor degeneration biomarkers. Overall, ^{1}H NMR spectroscopy was capable of profiling both CSF and VH in pigs, and of highlighting important quantitative differences between age groups or experimental groups, allowing to gain more knowledge regarding this important experimental species.

PB13

Iohexol as an intestinal permeability marker in a mouse model of intestinal inflammation

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Abstract
Assessment of intestinal mucosal integrity via intestinal permeability (IP) tests is a valuable diagnostic tool for screening and monitoring intestinal damage in patients and animal *in vivo* models. In mice, IP tests using iohexol have not been broadly applied and poses welfare issues, since animals must be housed in metabolic cages for prolonged times. The aim of this study was to standardize an *in vivo* IP test protocol in mice using iohexol and describe a time-course over 24-hour urine collection.

Male and female Rag1-/-mice (n=24) were injected with CD4+CD62L-CD44high effector-memory T-cells. Mice develop bowel inflammation, leading to weight loss. After 2,4and 5-weeks, a single dose of iohexol 10ml/kg was orally administered before animals were randomly placed in individual metabolic cages. Urine was collected after 2,4,6,12,15,18and 24-hours post-administration. Iohexol concentrations were measured using ELISA. Intestinal histological damage was scored in HE-stained tissue sections.

Urinary excretion of iohexol increased and peaked after 4-hours. Thereafter, iohexol concentrations declined progressively. A second peak was identified after 12-hours. A group-effect occurred at 2and 4-hours, and sex-group-effect at 12-hours. After 4-hours of housing in metabolic cages, more than 90% of mice delivered urine, reaching 100% after 12-hours.

The results suggest that Iohexol in urine increased during disease exacerbation with time. Urine collection 4-hours after iohexol gavage may be sufficient to evaluate IP in models of intestinal inflammation in mice. Therefore, housing of mice in metabolic cages for prolonged periods may be avoided.

PB 14

A non – invasive rat model of perinatal mild hypoxic brain damage

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Abstract
We aim to introduce a non-invasive perinatal mild hypoxic brain lesion in rats. Nineteen Wistar Han® (RccHan®:WIST) rats, (9 females and 10 males) were randomly divided into hypoxic and control group on postnatal day 1 (P1) when hypoxia was induced in a hypobaric chamber (Atm350mmHg, $pO_2$73mmHg, temperature $\approx$ 25°C) during 2 hours, while controls were kept in normal housing conditions. Behavioral tests were performed at P30 and P70 using the open field, hole board, social choice, and T-maze tests. Samples of brain tissue from adult animals (P105) were used for histochemical examination of the cytoarchitectonics (Nissl staining), interneurons (parvalbumin immunohistochemistry) and perineuronal nets (*Wisteria floribunda agglutinin*, histochemistry). After short-term perinatal rat brain injury, there were no disturbances in the brain macro-morphology or any other pathoanatomical consequence of the treatment. Also, treated animals had intact exploratory, anxiety-like and social behavior. Still, distinct changes in morphology, number, and distribution of the parvalbumin-immunoreactive neurons and perineuronal nets in different brain regions were observed. Moreover, treated animals displayed significantly impaired learning. In conclusion, the proposed rat model of non-invasive hypoxic brain lesion has indicated consistent disturbances in brain connectivity related to cognitive processes, mimicking perinatal mild post-hypoxia condition in humans. Further characterization and evaluation of the model, on molecular, cytological, and connectivity levels is needed.

PB15

Analysis of a rabbit cartilage defect model; Points of consideration

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Abstract
Rabbits are widely used in musculoskeletal research for the creation of cartilage defects on the distal femoral metaphysis. The rabbit cartilage defect model has been developed to simulate the human environment and conditions as a mid-step between *in vitro* testing and preclinical trials in translational orthopedics. Experimental bone defects are among others used to evaluate fracture repair, biomaterials, unfavorable wound environment, congenital musculoskeletal disorders, surgical techniques and fractures as complications of trauma, tumors, etc. As a result, it is important to develop specific guidelines for this animal model.

In a recent study, the development of hyaline to fibrocartilage through activation of the wnt/β-catenin signaling pathway was investigated through the creation of microfractures in femoral subchondral bone and lithium *per os* administration in New Zealand White rabbits of 16 weeks of age. The aim of this report is to indicate potential pitfalls in this animal model along with points of consideration regarding its reproducibility. Management and possible complications are also described. The purpose is to provide a resource for quick reference for the cartilage defect rabbit model and points of consideration, in order to maximize the value of similar future studies, in parallel to the model's welfare.

PB16

The rabbit as an animal model of bone defects

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Abstract
The New Zealand White (NZW) rabbit is widely used in orthopaedic research as an animal model for the treatment of bone defects arising from trauma, infection or neoplasia. Although autografts are the gold standard in clinical practice, restrictions on their applicability have led to the study of bone grafting substitutes.

The preferred anatomical sites for creating an experimental defect and evaluating grafts in the rabbit model are the calvaria, the mandible, the femoral condyle, the femoral greater trochanter and the proximal tibial metaphysis. The dimensions of a "critical-sized" bone defect (i.e. the smallest defect that cannot be spontaneously filled by normal bone tissue without intervention within the lifetime of the animal[1]) vary, depending, among other factors, on the bone site selected. Published studies report defect sizes of 15×15 mm in the calvaria, 8×8 mm in the mandible, 3×15 mm or 6x6 mm in the femur, 4.5×9 mm or 5×15 mm in the tibia. Some studies lack administration of analgesia due to its potential implication in the healing process, thereby compromising the rabbits' welfare. Appropriate analgesia, as well as minimum dimensions of defects, in parallel to the scientific objectives, are areas of consideration for improvements.

There is an increasing production of promising new biomaterials as well as their combinations that are being tested on bone defects of the NZW rabbit, which is an animal model that would benefit from the dissemination of up-to-date guidelines.

PB17

Canine models in biomedical research

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Abstract
The majority of dogs used in research, upwards of 75%, are estimated to be used in pharmaceutical testing, even though many scientists have concluded that they are poor predictors of drug effects in the human body. (More than 50% of all dogs are used in ''toxicological and other safety evaluations of products and devices for human medicine and dentistry and for veterinary medicine.) They are still used because regulatory authorities require that drugs be tested in both a rodent and a non-rodent species for toxicity, and the latter is often dogs, due to their ready availability, as well as their trusting and social nature and their anatomy and physiology are similar in many aspects to those of humans which makes them easy to handle. (The dog as 2nd species in EU legislation on chemicals, biocidal and plant protection products.) Dogs are also used in many other areas of biomedical research, including heart research, surgery, dental health and studies of hereditary diseases, in addition to research on the health, nutrition and behaviour of dogs themselves. Conditions in specific breeds like mast cell tumours in Labrador Retrievers, rage syndrome in English Springer Spaniels or inflammatory bowel disease in Boxers. The dog as a new ''natural model'' for human behavior: poly-morphism in the dopamine D4 receptor gene and the (hyper-) activity of dogs; which led to observations regarding human ADHD (attention deficit hyperactivity disorder).

PB18

Sex differences in sepsis model induced by cecal ligation puncture (CLP), in (Gal-KO) mice

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Abstract
Sepsis is a serious medical condition, caused by a fulminant immune response to infection and animal models to find new treatments are still necessary. CLP in 1,3-galactosyltransferase knockout (Gal-KO) mice was used to evaluate the model in our laboratory. CLP is the most frequently used model as it resembles the progression and characteristics of human sepsis.

15 males and 15 females were under CLP following published methodology (Rittirsch et al. NHI Nat Protoc 4:38, 2009; doi: 10.1038/nprot.2008.214) and analgesic protocol (ketamine, xylazine, buprenorphine) in order to validate the model in our laboratory. Mice were followed up daily according to a supervision protocol and established humane end-points based on body weight change, general appearance, behaviour, hydration and Mouse Grimace Scale. No spontaneous mortality occurred.

While no differences in female and male survival rate were observed at day 4 (40% in males vs. 50% in females), survival rate at the end of study at day 15 was higher in females than in males (50% vs. 20%).

Previous studies in other mice strains had indicated that females are more resistant to sepsis. Our study corroborates this and suggests that this difference is independent of mice strain. However, to avoid the use of only males in CLP studies, some adjustments (different number of males vs. females, severity of the surgery, etc) need to be evaluated to be able to study the influence of sepsis and its treatment in females as well as males.

PB19

Naturally occurring nosocomial Acinetobacter baumanii pneumonia in a congenic rat colony

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Abstract
Acinetobacter baumanii is an emerging pathogen in humans and causes an often-deadly pneumonia, and is associated with critical care and mechanical ventilation. The organism is highly resistant to many antibiotics and is transmissible via intranasal inhalation. A mouse model is the most common animal model used to study the pathogenesis of the organism and potential therapeutic treatments. However, these animal models have severe limitations, in that colonization with the organism is difficult, as is animal-to-animal transmission. In our congenic rat colony on a Wistar Furth background, we witnessed outbreaks of a fatal pneumonia. Symptoms included clinical and histopathological signs of a hemorrhagic suppurate bronchopulmonary pneumonia. Other less dramatic symptoms included weight loss, dystocia and early term abortions. Culture of lung tissue resulted in a pure culture of *Acinetobacter baumanii*. It is highly contagious between cage mates and thus, indigenous in this colony. Colonization of the organism in the lung and respiratory tissues was throughout the respiratory and lung tissues, even in animals without gross lung lesions. Dystocia, stillborn pups and early term abortions correlated with sepsis and dramatic weight loss. As perpetuation of this colony is vital to continue the congenic strain, cages with bedding were autoclaved to reduce dust, ancillary soft food and gel packs were added and antibiotic water provided on a weekly basis. As the only known naturally occurring animal model for this pathogen, it is the subject of intense investigation, including colonization, transmission and antibiotic resistance.

PB20

Staphylococcus aureus spontaneous struvitis in FVB male mice: a case series

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Abstract
Despite *Staphylococcus aureus* being considered an opportunistic bacteria and not included in the recommended infectious agents to monitor in laboratory mouse of the FELASA guidelines[1], we report a case series of spontaneous infection with *S. aureus* in colony mice. At the clinical inspection, 8 male mice (FVB; aged 10–13 months) shown signs of pain/distress (grimace scale scorev = 8, hunched position), body condition score = 2, ruffled fur, hypoactivity, high respiratory rate and shallow breathing. Mice were euthanized for welfare and necropsy revealed enlarged bladder filled with abundant urine and one white stone of approximately 0.3 cm diameter floating free, compatible with a struvite calculus. The microbiologic tests reported a positivity to *S. aureus*. The FVB colony was tested and a high prevalence of *S. Aureus* detected. *Staphylococcus* species are well-recognized sources of urease production and in cystitis in mice[2,3]. To avoid further clinical issues, our strategy was to decrease its prevalence by treating all mice with Amoxicillin in water[4] for two weeks. The colony was retested negative for *S. aureus*. Since we cannot eradicate *S. aureus* in our SPF facility, we consider fundamental to maintain its prevalence low by reinforcing environmental hygiene measures, a rapid identification thanks to the systematic tracking of clinical cases in the facility and a prompt action (diagnose, and antibiotic treatment) in case of clinical signs due to *S. aureus*. This will improve animal welfare and scientific outcome.

PB21

Characterization of Escherichia coli isolated in pigeons from an animal experimental facility in Brazil

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Abstract
Microbiological monitoring is an important tool to ensure the sanitary quality of laboratory animals. Pigeons are widely used in behaviour analysis research and may constitute a reservoir of potential pathogenic microorganisms. Commensal *Escherichia coli* strains may be precursors of pathogenic strains due to acquisition of antimicrobial resistance and virulence genes, leading to an increase in host's susceptibility to diseases and eventually alterations on experimental outcomes. The present study aimed to characterize phenotypic, genotypic and resistance-associated aspects of *E. coli* from cloacal swabs of healthy *Columba livia domestica* housed in an animal experimental facility at Psychology Institute, University of Brasília. Of the 27 samples analyzed, 21 (77.8%) showed *E. coli* growth. Regarding the sensitivity of *E. coli* to antimicrobials, streptomycin (42.8%) and tetracycline (52.3%) were the least effective drugs in vitro. All isolates demonstrated in vitro sensitivity to ampicillin, cephalexin, ceftazidime, cefotaxime, ciprofloxacin, gentamicin, nalidixic acid, nitrofurantoin and trimethoprim-sulfamethoxazole. Biofilm formation was evidenced in 85.7% of *E. coli* strains. The phylogenetic characterization of *E. coli* revealed that most of the isolates belonged to group B2 (47.6%), followed by groups A (38.1%) and D (14.3%). Although no multi-resistant isolates were found, surveillance of commensal *E. coli* in laboratory pigeons may help to minimize the spread of antimicrobial resistance and monitor for events that can affect animal's health and consequently experimental data.

PB22

A new Corynebacterium sp. (HAC2) that causes skin disease and dehydration in immunodeficient Mice

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Abstract
A novel Corynebacterium species was identified in NOD *scid* gamma mice with hyperplastic and hyperkeratotic dermatitis and a history of lethargy, dehydration, and death. Cultures and PCR tests were negative for *Corynebacterium bovis* and a novel coryneform bacterium was isolated. Portions of multiple genes of this bacterium were sequenced for the design of a diagnostic real-time PCR assay. Reference spectra from cultures were collected via MALDI-TOF mass spectrometry for specific identification of the novel Corynebacterium species. An experimental infection study was performed using nude (Hsd:Athymic nude-Foxn1nu), NOG (NOD.Cg-Prkdcscid Il2rgtm1Sug/JicTac), and ICR (Hsd:ICR) mice. The novel *Corynebacterium* sp. inoculum was applied to intact skin. At 4- and 6-wk post-inoculation (WPI), all oral and pelt swabs and most fecal samples collected from nude and NOG mice tested positive by bacterial culture and PCR tests. At 6 WPI, infected nude mice had moderately thickened erythemic skin with minimal keratin scale production and infected NOG mice were dehydrated with unkempt hair coats, thickened skin, and moderate keratin scale production. Histopathologic evaluation revealed the same hyperplastic skin lesions and gram-positive coryneform bacteria as in the clinical cases. None of the inoculated ICR mice were colonized or developed hyperplastic dermatitis. All sham inoculated mice tested negative by all tests at all time points. Thus, this novel *Corynebacterium* sp. is the cause of severe clinical disease in immunocompromised mice by colonizing the skin and inducing a hyperplastic dermatitis. The descriptive term hyperkeratosis-associated coryneform 2 (HAC2) is suggested for this bacterium.

PB23

Successful treatment of Pasteurella pneumotropica mastitis in Peromyscus polionotus with enrofloxacin in water

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Abstract
Eighty *Peromyscus* spp. mice (40 *P. maniculatus* and 40 *P. polionotus*) were received from the Peromyscus Stock Center in fall 2017 with a clear health report and no history of clinical issues in the colony. Mice were housed in IVC with both RO treated automatic water and RO water in bottles available. In March and April of 2018, two breeding *P. polionotus* females presented mammary chain swelling and lameness responsive to antibiotics. Necropsy and culture confirmed bacterial mastitis and blood culture positive for *Pasturella pneumotropica*. Exhaust Air Duct (EAD) PCR confirmed *P. pneumotropica* in July 2018. Until that time, all affected mice were imported. However, as *P. pneumotropica* is transmitted in utero, it was expected that offspring would also be susceptible to infection. When an offspring female *P. polionotus* presented with similar clinical signs colony treatment was elected. All mice in the colony were treated with 0.5 mg/mL enrofloxacin in drinking water for two weeks. Subsequent EAD PCR testing performed in Sept 2018 and Dec 2018 were negative for *P. pneumotropica*, and no further clinical cases of mastitis have been observed.

PB24

Immunologic response to Demodex musculi in Swiss Webster, BALB/c and C57BL/6 Mice

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Abstract
Historically, detection methods for *Demodex musculi* were unreliable and testing was rarely performed as its presence in laboratory mice went under-recognized. While infestations are clinically inapparent in most mouse strains, *D. musculi* burdens are increased and clinical signs have been reported in select immunodeficient mouse strains. However, the parasite's impact on the immune system of immunocompetent mice is unknown. We characterized mite burden and immunologic changes in naïve Swiss Webster (outbred), C57BL/6NCrl (Th-1 responder), and BALB/cAnNCrl (Th-2 responder) mice following exposure to *Demodex*-infested NSG mice. Age-matched mice of each strain (n = 5) were euthanized 14, 28, 56, and 112d post-exposure. Mite burden was determined by PCR and skin histopathology; B cell, CD4 and CD8 cell counts, and T-cell activation markers (CD44, CD25, CD69, Ly6C) were evaluated using flow cytometry; complete blood counts were performed, and serum IgE levels were measured by ELISA. Most infested animals developed diffuse alopecia by 112d and both BALB/c and C57BL/6 mice had significant alterations in IgE levels, T cell populations as well as certain innate immune cells compared to uninfested animals at various time points. These findings aligned with the inherent immunophenotypes of each strain, with BALB/c mice mounting a more effective response which significantly reduced mite burdens. These findings demonstrate a need for for *Demodex musculi* surveillance in immunocompetent mouse colonies as the resulting immune perturbations that result from infestation may impact the use of infested mice in select studies.

PB25

Animalstudyregistry.org – A federal database for preregistration of animal research

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Abstract
The reproducibility of results gained from animal experiments and their extrapolation to humans are intensively discussed. Reporting bias, HARKing (Hypothesizing After the Results are Known) and p-hacking have been identified as major factors contributing to the reproducibility crisis. Hence, greater transparency of animal experimentation is requested by the public as well as by the scientific community. As a potential countermeasure to reduce selective reporting, the German Centre for the Protection of Laboratory Animals (Bf3R) at the German Federal Institute for Risk Assessment (BfR) has launched a new preregistration platform for animal studies, Animal Study Registry (www.animalstudyregistry.org).

The registry is designed for exploratory and confirmatory studies in the field of fundamental and preclinical research. The registration form serves as a check list for the researchers, helping them to plan their study thoroughly by asking detailed questions concerning study design, methods, and statistics. With the registration in Animal Study Registry the study receives a digital

objective identifier (DOI) which marks the study as intellectual property of the researcher. The long embargo period of 5 years is a distinctive characteristic of Animal Study Registry. During the embargo period the visibility of the study is restricted to a short summary. As a scientifically independent federal institution, the BfR provides continuity and data security.

Registering a study in the Animal Study Registry proves the researcher's commitment to transparency and data quality to reviewers and editors, to third party donors, and to the general public.

PB26: Withdrawn

PB28

Human all too human: The limits of scientific integrity in addressing suboptimal animal-to-human translation

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Abstract
Ethical justification of animal experiments often starts with the claim that their results can be translated to human diseases. The debate on translational success rates seriously undermines this justification. To explain and get a grip on low success rates, flawed methodology and inadequate scientific practices are mentioned. Therefore, scientific integrity seems a relevant framework to address problems of translational success.

We will question the relevance of this framework. First, it risks only considering problems of fraud and fabrications, thereby ignoring other dubious research practices. Second, and more important, the integrity framework seems to view scientists rather rationally, i.e. scientists know what is right, but consciously act otherwise. However, a number of scientific integrity problems related to low translational success, (e.g. bias in analysing data) also happen unconsciously.

These problems cannot be changed by increasing scientists' rational thinking. Even the scientist is ''human, all too human'', and vulnerable to bias. Psychological research shows that emotional drives unconsciously steer the ratio to generate all kinds of arguments. As an alternative, we propose the framework of ''indirect self-control''. This framework acknowledges the vulnerabilities of scientists and stresses the need to formulate self-regulated measures to improve scientific practice. It implies that scientists limit the possibility of bias in their experimental design, e.g., by incorporating blinding to prevent expectation bias, or by supervision by others or preregistration (e.g. at www.preclinicaltrials.eu) that limits the possibilities for cherry picking.

PB28

Planning, conducting and reporting farm animal research

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Abstract
Improvements in farm animal welfare have mainly focused on those kept under farm conditions, and may be less applicable when they are housed in the laboratory. Confining animals under regulated conditions of space, ambient temperature and lighting conditions may cause considerable stress. Their amenability to physical restraint will also be less, which may stress the animal and increase the risk of injury. Meeting standards of hygiene and personal protection which are commonplace in rodent facilities may prove challenging. Specific problems

arise with farm-sourced young animals which have to be weaned before transport, or brought to the facility with their dams.

There are other areas of concern: 1) there is a paucity of information on pharmaceuticals, including analgesics (many drugs are unlicensed for use in some species and age groups) there is little information on signs of pain after experimental surgeries; 3) the use of analgesics after noxious procedures is under-reported and probably inadequate; 4) the expense of large animal housing promotes a tendency to minimize acclimatization; 5) farm-sourced animals may *never* acclimatize to the laboratory environment.

There are fewer recommendations and guidelines for the care and use of farm animals. Norecopa has organized an international consensus meeting and built guidance into the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines website. There are no grounds for denying farm animals the same standards of protection that are provided for other species. This will also increase external validity when farm animal models are used in translational research.

PB29

Standardization, reproducibility and high-quality data collection from pre-clinical, behavioral, in-vivo research

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Abstract
A number of clinical trials fail, despite promising pre-clinical data. Therefore, standardization, reproducibility and high data density play a key role in in-vivo experimentation. That is why the improvement of the methodology of pre-clinical data collection is of great importance.

The aim of our pilot study, within the project no. ATCZ40 (Stimulation innovation in the Czech-Austrian border region through the use of available synergies in research infrastructures) is to set quality standards to achieve comparable, reproducible and data rich results. In one benchmarking study, similar experiments, with the use of the same systems, are conducted in two different behavioral laboratories – in Austria and in the Czech Republic. Three pilot projects: 'Development of cognitive function pipeline', 'Development of a pipeline for depression/anxiety-like behavior', 'Motorater gait analysis' are performed simultaneously by the aforementioned core facilities. Their outcome will allow us to provide a validated methodology of behavioral testing to future users of both core facilities and the field en masse.

All research in the area of behavioral testing will benefit from this project: researchers, who will gain access to optimized and standardized methodologies; Lab Animal Science community, who will be able to highlight significant issues and bottlenecks in behavioral testing; the pre-clinical research community, since validated data and experiment reproducibility will help to overcome the attrition problem in translating results into the clinical arena. Moreover, sharing of knowledge will improve and serve the future quality assurance.

PB30

Increasing external validity of your animal model: Benefit of controlled sources of variance

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Abstract
Statistical power is the probability of detecting true effects exerted by independent variable(s) on a dependent, measured variable. In simpler terms, it represents the probability of finding a difference between groups when the difference is really present. Power depends on sample size, but also on the standard deviation of the measured dependent variable within each group. In many biological experiments, sample size is a limiting factor, and power is gained by reducing within-group variability. Typically, experiments are run on mice of the same strain, age, and sex. This kind of design has two major drawbacks: 1. Strongly limits external validity (i.e. generalizability) of the results; and 2. Entails a considerable waste of animals that are often produced yet not included in the study. We show here the advantages of increasing the size of experiments by inclusion of female animals, or different inbred strains, or multiple batches of animals. The inclusion in data analysis of proprietary factors such as sex or strain, or technical factors such as batch of animals, can allow considerable increases in external validity, with negligible loss of power, by separating the variance linked to the experimental factor from the variance caused by other factors. The small loss of power linked to the addition of factors is largely compensated by the gain of power associated with the possible inclusion of more samples, otherwise wasted. Inclusion of female animals and designs involving different inbred strains have much larger external validity and should be privileged in translational, preclinical studies.

PB31

Development of automatic micromanipulation system for genetic modification of mice

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Abstract
Many transgenic, knockout, and knock-in mice have been generated using micromanipulation techniques. However, use of a conventional micromanipulator requires a high level of technical skill. Therefore, we have developed the Integrated Automatic Embryonic Manipulation System (IAEMS), which can be used to easily perform micromanipulation. The IAEMS consists of two XYZ manipulators, an XYZ microscope stage, and two pumps, which are motorized

and controlled automatically by software. The XYZ manipulator and pump hold, release, and inject embryos using pipettes. The XYZ microscope stage adjusts the position of the field of view under the microscope. The IAEMS is also equipped with actuators to perforate the zona pellucida and cell membrane. In this experiment, plasmid DNA solution (pEGFP-N1) was injected into mouse pronuclear oocytes and embryonic stem (ES) cells were injected into blastocysts using IAEMS. The survival rate after motorized and automated DNA injection was 96% ($n = 231$) and four (2%) transgenic mice were obtained. With use of a piezoelectric actuator with the IAEMS, the survival rate was 96% ($n = 154$) and four (3%) transgenic mice were obtained. In ES cell injection using IAEMS, chimeric mice were obtained from embryos injected with ES cells, and germ line transmission derived from ES cells was further confirmed. These observations suggested that it is possible to perform highly repeatable injection operations with simple training using IAEMS. We are currently attempting to develop a fully automated micromanipulation system for genetic modification of mice.

PB32

Stability of cryopreserved mouse embryos

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Abstract
Genetically modified mice are unique mutants exhibiting a major scientific potential. Small populations, continued danger of loss, limited breeding success due to an inbred genetic background, the need to keep them in stock even if they are out of experimental use, the breeding surplus, and frequent interchanges between different facilities are issues when dealing with these mutants. Cryopreservation of pre-implantation embryos is a valuable alternative to breeding. Strict assessments are mandatory. Aim of the cryopreservation is the secure recovery of the line also after years. Following sufficient cryopreservation the breeding of a line can be discontinued.

The common assessment of viability is the morphological investigation of frozen/thawed embryos after revitalization before subjected to an embryo-transfer to recover the line, possibly *in vitro*-cultures to elucidate the developmental capacity. We have revitalized 30.000 randomly selected cryopreserved eight-cell-embryos with different mutations and different genetic backgrounds, stored up to 22 years. >90% were morphologically intact, about 1000 of these revitalized embryos were cultured overnight exhibiting the capacity of >90% to develop to blastocysts.

To better understand the loss, we investigated signs of cell death by staining 2500 revitalized cells stored between ten and 22 years. <10% were propidium-iodine positive, i.e. >90% were viable. About 5% of the cells in blastocysts showed signs of apoptosis, the same as published for ''fresh'' blastocysts. Taken together, cryopreservation of pre-implantation embryos and their long-term storage in LN2 is a valuable alternative to breeding mouse mutants also contributing to animal welfare and the ''3Rs''.

PB33

Challenges in thermoneutrality management and experimental design using fat deficient mouse model

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Abstract
Housing temperatures can have a large impact on the physiology of experimental animals. Laboratory mice are usually kept under thermoneutral conditions (22°C) in order to model human metabolic rate. However, these conditions create challenges in survival, breeding and experimental design in adipose tissue-deficient mouse models. Here, we present data using a new fat deficient mouse model which lacks PPARg, a transcription factor which plays a crucial role in adipogenesis and lipid metabolism. To circumvent the embryonic lethality of its germline deletion we generated Sox2Cre-Pparg (floxed) mice which could survive but had phenotypic abnormalities, high newborn lethality and high sensitivity to environmental conditions (temperature, humidity). We could improve the survival and the colony size by keeping mice on thermoneutral temperature (30°C) that allowed further study of their phenotype. We find that PPARg KO mice suffered liver steatosis, organomegaly, insulin resistance and completely lacked white and brown adipose tissues. Calorimetric and metabolic parameter measurements with the Oxymax-CLAMS system showed that Pparg KO mice had higher O_2 and CO_2 production, higher food and water intake but showed lower overall activity. Interestingly, the lack of adipose tissue affected thermoregulation markers of these mice with the effect being more severe in older animals. In addition, lipidomic analysis showed an inverse correlation of free fatty acid, triglyceride, sphingomyelin, phosphatidilcholine and phosphatidilethanolamine acummulation in the liver and the muscle, respectively, in PPARg KO mice. Finally, subcutaneous fat tissue transplantation into PPARg KO mice could improve metabolic parameters and thermoregulation.

PB34

How habituation can improve the reliability of experimental data – A retrospective analysis

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Abstract
The prior habituation to the test situation is a common practice to minimize putative external stress factors and to improve the reliability. Published empirical knowledge regarding optimal number of habituation trials and the effect of habituation on repeatability of test results is missing. Therefore, this study aimed to elucidate retrospectively the effectiveness of habituation of male mice to an Open Field (OF) arena and Incremental Hot Plate (IHP).

For a period of six consecutive days, male mice (C57BL/6J, Balb/cJ, 129S1/SvImJ) six to seven weeks old become habituated to an OF arena (ø 30cm) and IHP (at 35°C). Individual mouse behavior was recorded and assessed concerning locomotion (for OF) and explorative behavior (for IHP). To test the reliability of the data, a repeatability analysis was conducted with the software R.

From the second day of habituation onwards a significant reduction of all measured behaviors was present. With the exception of the first repeatability measurement of locomotion (day 1–3), all repeatabilities were significant. As from the second grouping (day 2–4), a stable repeatability value for all parameters is present, meaning that around 60% of the variance of the data can be explained by individual differences between mice. Mouse strain-dependent influence seems to be subordinated.

Preliminary data indicate that prior habituation led to a significant increase of repeatability and subsequently improve the reliability of experimental data. Additionally, a habituation period of three to four days appeared sufficient and thus minimizing the experiment's duration for the animal.

PB35

Published concordance rates between animal experimental and human trials – A systematic scoping review

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Abstract
Background: Biomedical research investigates human pathophysiology to develop new cures, but drug development is challenging. Candidate drugs that are successful in animal experiments often fail in clinical trials. One explanation is that the concept of animal-to-human predictability is fundamentally mistaken; this concept was never formally tested. An alternative explanation is that animal models can be predictive for humans, but that the quality of the experiments needs to improve to increase predictability. Both perspectives are currently defended by scientists arguing that translational success rates are (too) low.
Objectives: Several authors have addressed translational success rates. We performed a systematic scoping review to collect and describe the available aggregated quantitative data. We define successful translation as correspondence of results from animal experiments with human trials.
Method: Comprehensive searches in Pubmed and Embase were supplemented with reviewing reference lists and contacting content experts. We included papers that quantitatively compared the results of studies including at least 2 species with one being human, providing quantitative information on translation. The full protocol is available on www.syrcle.nl (Menon et al. 2017).
Results: Our searches retrieved 2649 references. After title-abstract screening, 160 remained, after full-text screening, 26. From the reference lists, we retrieved 60 more relevant papers. Content experts contributed 35 more.

Included papers comprise studies explicitly addressing translational success rates, but also correlational and meta-analyses including both human and animal data. The range of observed translational success rates was 0-100%. A full analysis of the included papers will be presented at the conference.

PB36

Development of a necropsy protocol for newborn mice

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Abstract
Studying pup mortality is challenging, as dead pups are often eaten by the older mice before being found. As a result, the literature lacks specific guidelines for the appropriate necropsy of newborn cadavers. Therefore, we designed a necropsy protocol to inspect newborn mice for major malformations, presence of lesions in any of the major organs, as well as viability indicators, including presence of milk in the stomach and proof of breathing after birth. The latter was obtained through a floatation test using lung and liver fragments. If the lung floated in water (i.e.presence of air) it was considered that the pup breathed before dying. The liver was used to distinguish the breathed air in the lungs from gases from tissue decomposition. Pups originating from C57BL/6J breeding trios (n = 37) and pairs (n = 25) under SPF conditions were found dead and evaluated, while the lung floatation test was performed in 52 of them.

Dead pups had an average weight of 1.24 ± 0.21 g, aged 0.34 ± 0.51 days, and were 2.61 ± 0.21 cm long.A total of 27 pups breathed before dying and their lungs had visible air bubbles and a darker coloration contrasting with the pale pink lungs which sunk. Only 7% of the pups who breathed had milk in their stomachs, whereas none of the animals considered not to breath had milk on their stomachs. No major malformations were observed. Results suggest that it is possible to determine if pups breathed and ate before dying, which is a starting point to further augment newborn post-mortem analysis.

PB37

A novel semi-solid pill for stress-free voluntary oral drug administration in experimental rodents

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Abstract

During compound screening and drug development, long-term oral drug administration to experimental rodents is often required. Oral gavage, a straightforward drug dosing technique, is not suitable for extended treatments considering the recurrent traumatic complications (gastroesophageal injury) and physiological distress (corticosterone levels alterations) that frequently bias experimental design outcomes. These reasons create a challenge for preclinical drug assays and stress-free/metabolic-inert alternatives of oral drug administration are warranted.

Herein, it is presented an innovative semi-solid pill optimized to overcome aforementioned drawbacks. After a brief training period, C57BL/6 mice submitted to a chronic oral administration protocol (50 days) displayed a high index of voluntary acceptance of empty- and drug- (*e.g.* sitagliptin) incorporated vehicle in both healthy and CNS-diseased states. This protocol operates in a pair-housed animal housing fashion, allowing animal socialization throughout entire protocol. At the end of experiments, a normal neurobehavioral phenotype (anxiolytic, memory, locomotion parameters) was recorded. Moreover, this new methodology proved to be safe, preserving serum metabolic (glucose, triglycerides, total cholesterol), hepatic (albumin, total proteins) and renal (urea, creatinine, uric acid) parameters along with normal ileum contractility. Remarkably, coherent sitagliptin (±10 μg/ml) plasma levels were detected, along with a robust decrease (±80%) on the activity of its target (dipeptidyl peptidase-4), unequivocally proving *in vivo* drug efficacy.

Overall, this innovative approach may enclose a breakthrough advance for translational studies in scientific and pharmaceutical fields, providing a reproducible, efficient, metabolic inert and stress-free alternative for voluntary oral drug administration, with expected improvement on the data feasibility.

PB38

The bias in the publication of scientific studies

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Abstract

Science is often romanticised as a flawless system of knowledge building, where scientists work together to systematically find answers. Ratherth an approaching a research question in a systematic manner, it seemsthatscientistsare encouragedto pursuenon-linearlinesof investigation in searchof significance, and many tuck away negative findings (the "file-drawer" effect) and focus on theirpositive outcomes. Hours of intense work and significant monetary expenditures, animals become a victim of the ¨file drawer effect¨. This behaviour likely stems from an ever-heightening hurdle that scientists need to jump: **high publication output with a high citation rate in order to win competitive grants to drive their research, move up the rungs and pay the bills.:** It has recently been reported that the conduct of research is ¨often inadequate¨, due to incomplete reporting of relevant information. This leads to a paramount of waste of financial resources and poor publication quality. Failure to describe research methods and to report results appropriately therefore has potential scientific, ethical, and economic implications for the entire research process and the reputation of those involved in it. Most of scientific reports do not use correct randomization or blinding to reduce bias in animal selection and outcome assessment.

PB39

Gut microbiome in diet-induced type 2 diabetes mouse model – Does the sex matter?

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Abstract

Biomedical research is often performed using only one sex (generally male) of animals for experiments, though the results are extrapolated to both sexes without thorough justification. This might cause harmful effects on the neglected sex and substantial economic loss. Growing evidence suggests an important role of gut microbiome in type 2 diabetes (T2D) therapy with anti-diabetic drug metformin. However, existing animal studies focusing on this have several drawbacks – including inefficient experimental design and involvement of males only, rendering the interpretation of results inaccurate. With the aim to evaluate the effect of metformin on microbiome composition of different sites of gastrointestinal tract we have performed an *in vivo* study in which C57BL/6N mice of both sexes, divided into 24 experimental units (cages) were included (in 3 replicates, 72 in total). T2D was induced by high-fat diet (60% kcal from fat) in half of the cages and half of animals received metformin therapy. The duration of the experiment was

31 weeks and it had randomized block design with three-way factorial treatment arrangement forming eight groups. During the study both individual and pooled fecal samples were collected every week; plasma samples were collected at different time points; and various metformin-target tissue and gut microbiome samples were obtained after euthanasia. Initial results from the metagenomic analysis of changes in gut microbiome composition in various intestinal segments in context of T2D manifestations and metformin treatment emphasizing sex-related differences will be presented substantiating the importance of inclusion of both sexes in animal studies where appropriate.

PB40

A comparison of microbiota variation of mice housed in IVC versus open top cages

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Abstract
This preliminary study was conducted to understand how different approaches to housing laboratory mice might influence the microbiota of the animals. 18 C57BL/6J (JAX™ Mice Strain) female mice at 8 weeks of age were sourced from a commercial supplier and divided randomly into two groups. Nine animals were housed in open top cages, and nine animals housed in individually ventilated cages (IVC's), both in 3 cages of three animals. All animals received the same food, water, environmental enrichment and bedding. During a 12-week period, individual faecal samples were collected at 8, 12 and 20 weeks of age with an additional caecum sample at termination (week 20), DNA was extracted and subjected to 16S rRNA analysis.

The results not only show an increase in the total number of microbiome of the animals housed in open top cages when compared to the IVC's but also differences in the community structure over time. Recently there have been many publications discussing the relationship between animal studies and microbiome and how this might affect results especially in the field of metabolic and immune research. This experiment not only demonstrates the need to carefully consider methods of housing rodents with respect to susceptible experiments but also the supplier, acclimatisation period and age of the animal in order to reduce experimental variation.

PB41

Hygienic condition in animal housing environment influences gut microbiota of mice for colitis development

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Abstract
Changes in environmental conditions and microbial status of experimental animals may influence disease progression of animal disease models. Some strains of intestinal bacteria are strongly associated with inflammatory bowel disease (IBD). Helper T lymphocytes are not only the key players in mediating host defense against wide variety of pathogens, but they also contribute to pathogenesis of many immune-related diseases. Th1 and interleukin-17 secreting T helper (Th17) cells play a critical role in inflammation and autoimmunity. The T cell transfer model of colitis is the most widely used model to dissect the induction and maintenance of chronic inflammation mediated by T cells. We studied how housing condition in different animal facilities influences gut microbiota of mice for development of colitis in a T cell-dependent manner. We applied adoptive transfer of naïve T cells to lymphopenic Rag-/- mice and monitored the development of colitis. Mice maintained in an animal unit without detectable pathogens developed mild disease. The same mice were then transferred to an animal unit where pathogenic agents such as *Helicobacter spp.* and murine norovirus detected during routine health monitoring. The microbial status and the gut microbiome of Rag-/- mice detected by routine health monitoring and 16S rDNA sequencing, respectively. Changing mice to pathogen-positive condition led to development of more severe colitis in this disease model. Animal health monitoring combined with next generation sequencing to detect changes of microbiota in mice may help to understand which pathogens in an animal unit are critical for development of T cell-dependent colitis.

PB42

Adaptive immune response in SPF and non-SPF BALB/c and C57BL/6 mice

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Abstract
To compare the adaptive immune response in mice with different sanitary standarts, splenocytes from SPF and non-SPF BALB/c and C57BL/6 mice were obtained, stained with different antibodies and analyzed by flow cytometry. Non-SPF BALB/c mice did not show any variation in the frequency of T (CD4+ and CD8+) and B lymphocytes when compared to SPF animals. However, when the expression of the activation molecules was evaluated, CD4+ T lymphocytes showed increased expression of CD28 and CTLA-4, whereas CD8+ T lymphocytes had a decreased expression of CD69 and CD25. B lymphocytes of non-SPF BALB/c mice showed increased expression of CD80 and CD69 and decreased expression of MHCII when compared to SPF animals. In non-SPF C57BL/6 mice, there was an increase in the frequency of CD4+ T lymphocytes and decrease in the frequency of CD8+ T lymphocytes in relation to SPF animals. In addition, CD4+ T lymphocytes showed increased expression of CD28, whereas CD8+ T lymphocytes had increased expression CD28 expression and decreased CD25 expression. There was no difference in B lymphocytes

frequency, but in the non-SPF C57BL/6 mice, the expression of MHCII, CD80, CD86 and CD69 was increased in these cells. The results show that the health status can affect the adaptive immune response in the strains studied.

PB43

Analysis of serum cytokines in SPF and non-SPF BALB/c and C57BL/6 mice

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Abstract

Several evidences show that experimental results can be significantly influenced by the state of health and environmental conditions of breeding and maintenance of the laboratory animals. To compare the cytokine profile of mice with different sanitary standards, sera from Specific Pathogen Free (SPF) and non-SPF BALB/c and C57BL/6 mice were collected and used to determine the serum levels of IFN-g, TNF-a, IL-17A, IL-12p70, IL-4, IL-6, IL-1b and IL-10 by flow cytometry. The SPF mice were certified according to recommendations of the International Council on Laboratory Animal Science (ICLAS). Non-SPF BALB/c mice were infected with MHV, PVM, MNV, MPV, ROTA, *Mycoplasma pulmonis*, *Syphacia muris* and *Tritrichomonas muris* and non-SPF C57BL/6 mice were infected with MHV, PVM, TMEV-GDII, REO-3, *Mycoplasma pulmonis*, *Syphacia muris* and *Tritrichomonas muris*. The results showed that non-SPF BALB/c mice had elevated serum levels of IFN-g (0.0 fg/mL *versus* 609.1±179.1 fg/mL) and TNF-a (2341 ± 918.3 fg/mL *versus* 7433 ± 2103 fg/mL) in relation to SPF mice of the same strain. On the other hand, non-SPF C57BL/6 mice showed elevated serum levels of IFN-g (0.0 fg/mL *versus* 607.5 ± 189.0 fg/mL), TNF-a (3489 ± 842.4 fg/mL *versus* 12794 ± 1675 fg/mL) and IL-17A (0.0 fg/mL *versus* 1774 ± 247.2 fg/mL). Together, the results show that the health status impacts on the cytokine profile of mouse strains that are commonly used in rodent-based research.

PC1

Study of an anesthetic mixture of medetomidine, midazolam, and butorphanol in rabbits

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Abstract

Objective: An anesthetic mixture (MMB) of medetomidine (MED), midazolam (MID), and butorphanol (BUT) has been used in mice and rats. However, there is little information regarding its effects in rabbits. The purpose of this study was to investigate anesthetic effects of MMB and antagonism by atipamezole (ATI) in rabbits.

Methods: Six male Japanese White rabbits were intramuscularly (IM) injected with MED: 0.15 mg/kg, MID: 1.0 mg/kg, BUT: 1.5 mg/kg, and atipamezole (ATI): 0.75 mg/kg. We measured 6 reflexes (body righting reflex, corneal reflex, and withdrawal reflexes of 4 legs pinched by hooked forceps) every 5 minutes (min) after MMB administration. The first time when all reflexes were disappeared, we decided it was a starting time of surgical anesthesia. The duration that all reflexes were disappeared, we decided it was a surgical anesthetic duration. The recovery time was decided when all reflexes were recovered. We also measured O_2-saturation, heart rate and respiratory rate every 5 min after MMB injection. Thirty min after administration of MMB, ATI was injected and we measured recovery time. After administration of MMB, blood was collected for biochemical testing.

Results and Discussion: After administration of MMB, surgical anesthetic duration was 25.8 min and recovery time was 111 min. O_2-saturation decreased lightly. Heart rate and respiratory rate decreased but stable. After ATI injection, recovery time was 17.5 min. After MMB injection, blood glucose was elevated. Overall, we found MMB to be a useful anesthesia for rabbits.

PC2

Could we refine a protocol of short anaesthesia in laboratory mice?

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Abstract

Intraperitoneal injections (i.p.) have been previously shown to be stressful to laboratory animals, however i.p. anaesthesia, without induction with inhalants is most commonly used in laboratory mice. This study evaluated the effect of two general anaesthesia (GA) protocols i.e. with and without induction with isoflurane on fear response in laboratory mice. The effect of the GA protocols was assessed based on the volume of urine present in the urinary bladder, as it was previously shown that urination is one of physiological responses to fear in rodents (Antoniadis and McDonald, 2001). The samples were obtained from 56 animals destined to be sampled under GA without recovery in accordance with Animals Scientific Procedures Act 1986, Project Licences issues by the Department of Health, NI. Twenty-four animals were anaesthetized withi.p. injection of ketamine + xylazine(10:1, 1.2 ml/kg LBM), while 32 animals were first induced with 5% isoflurane (anesthetic chamber) until loss of righting reflex observed, which was followed by i.p. ketamine + xylazine (10:1, 1.2 ml/kg LBM).

The results showed that animals' response to the two anaesthesia protocols differs significantly, indicating that the induction with inhalant (isoflurane) prior to i.p. anaesthesia may offer a refinement of benefit to animal welfare. However, since the anaesthesia protocols tested in this study were applied without recovery, the findings of this study need to be evaluated for protocols with

recovery allowing for the assessment of possible sensitisation/habituation of animals.

PC3

Behavioral testing and postoperative care in an ovine stroke model

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Abstract
Causes and treatment methods for stroke are studied in numerous small and large animal models. Readout parameters for monitoring experimental treatment success include magnetic resonance imaging, positron emission tomography, histology. Moreover, behavioral tests for assessing functional deficits after stroke are very common in rodents.

However, such tests are less developed and less frequently used in large animals and especially in sheep. Here we present a simple but reliable neurological test for sheep already proven effective in a number of studies. This test was recently complemented by a cognitive test for sheep. We investigated whether cognitive deficits after stroke are measurable, and whether the cognitive performance is generally affected by stroke. To this end, the learning behavior was examined in connection with feeding behavior. Therefore, differently colored, varying bins were used for feeding after habituation to a white bin. Failed attempts (looking in false bin) and time to reach the right bin and time to feed from a fixed starting point were recorded. The cognitive test contains several trainings and test periods. It was first established with healthy sheep and subsequently used with stroke animals.

Importantly, animals have to be effectively relieved from confounding factors such pain and isolation to obtain meaningful test results. We therefore report a pain management system for stroke sheep with Flunixin and Butorphanol.

PC4

Evaluation of two analgesia protocols on reproductive performance and pain management after embryo transfer

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Abstract
Analgesia is often considered as a confounding variable that may disrupt the performance of recurrent protocols such as embryo transfer. The impact of analgesics in this field is still a matter of debate especially when it comes to NSAIDs, supposed to perturb the foetus implantation due to their intrinsic mecanism of action on prostaglandins.

In our double-blinded placebo-controlled study, we evaluated the effect of 2 analgesia protocols on the procedure of embryo transfer in mice. 96 Crl:CD1(ICR) mice between 8 and 12 weeks were implanted under ketamine/xylazine anesthesia with 27 different strains of transgenic embryos and randomly allocated to the placebo group (n = 64 – NaCl 0,9%), the buprenorphine group (n = 16 – 0,1 mg/kg SC once) or the buprenorphine + meloxicam group (n = 16 – respectively 0,1 mg/kg SC once and 5 mg/kg PO in water during 2 days).

After the surgery, mice were observed daily and the clinical state, the water consumption and the Mouse Grimace Scale score were assessed during 2 days. The reproductive performance including gestation, birth and weaning rates were also recorded.

Results show no statistically differences between the 3 groups either in reproductive performance or in pain management efficiency which suggests that the analgesic coverage of ketamine/xylazine anaesthetic protocol is sufficient for this low traumatic surgery. The use of meloxicam had no adverse effect on pregnancy rate but slightly reduced water consumption, probably due to aversive behavior. This study is the first to our knowledge to assess the pain management efficiency in embryo transfer.

PC5

Description of vital parameter trends during long-term anaesthesia for preclinical non-survival studies in pigs

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Abstract
In context of different preclinical in vivo drug-testing and bioavailability studies in laboratory pigs with the aim to improve the performance of an existing minimally-invasive sampling method, a balanced partial intravenous anaesthetic protocol was used. General anaesthesia for these long-term and non-survival procedures consisted of propofol and fentanyl continuous rate infusions and inhalation anaesthesia with isoflurane. Pigs (sus scrofa domesticus) were in dorsal recumbency without any change in position and their lungs were mechanically ventilated during the whole experiment. The recorded vital parameters (heart rate, oxygen saturation, respiratory rate, endtidal CO2, invasive blood pressure, body temperature) and ventilator settings of seven pigs were evaluated retrospectively and are presented here. The duration of general anaesthesia was 15 hours and no severe cardiorespiratory complications were observed. Together with the recent findings from the literature these data can lead to further improvement of study conditions for preclinical trials in the context of drug development and approval. The presented balanced

anaesthetic protocol could be successfully used for preclinical studies which require a stable and safe long-term anaesthesia for minimally invasive procedures in laboratory pigs.

PC6

Swine post-operative monitoring and human end-points in preclinical implantable cardiovascular medical devices evaluation

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Abstract
Swine are considered to be one of the best animal models for the development and validation of implantable devices in cardiothoracic surgery. The preclinical evaluation of the cardiac medical devices is stated as a severe in classification of procedure by Annex VIII of the Directive 2010/63/EU as these procedures may cause different degrees of pain, suffering, distress or lasting harm and could be experienced by an individual animal during the course of the procedure leading eventually to animal's death. For this reason special surveillance procedures of animals are necessary in order to achieve an immediate intervention by operators. A peculiar problem of these experimentations might be a worsening of the animal state of health within a few hours causing the subsequent death of the subject. The subject's suffering and death are not only an ethical issue, but they also cause a severe loss of data necessary for a successful project. A daily monitoring system is outlined through a check list integrated with the human end-points. The evaluation of the collected data every eight hours enables the veterinary staff, coordinated by the Designated Veterinarian, to intervene promptly applying the appropriate therapies. In the event that the human end-point is reached it is possible to make a final follow-up under general anesthesia before the suppression of the animal.

PC7

Hypothermia prevention in the small animal post-surgical recovery cage

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Abstract
Hypothermia is a major influencer in surgical intervention and post-surgical care in small laboratory animals. Therefore, prevention of hypothermia is an important issue to reduce harm and distress in post-surgical animals and contributes to refinement (3R) of methods and good laboratory practice. In daily routine of animal research facility supervision, different and sometimes insufficient solutions are found. Furthermore, depending on health status, provision of a heating source/prevention of hypothermia should last until full recovery and the animal should be able to withdraw itself from the heating source to prevent harm by overheating and burns. Here an (cost) effective solution for a thermal cage is presented usable in intervention and housing of small animal rodents providing a zoned temperature gradient in the cage with the option to be supplemented in existing housing situations. This is achieved by a power-limited flexible film heating-mat between the cage bottom and a disposable cage insert currently available for European type II L and III H cages. Temperature limit and power supply are thermostat-controlled and the system is therefore considered safe for continuous use. Because of its simplicity, it promises a much better acceptance that may encourage manufacturers to supply more types of disposable cage inserts a fact still limiting availability for all cage systems on the market.

PC8

Comparison of long-acting opioid with standard buprenorphine and NSAID in post-surgical pain in minipigs

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Abstract
A number of long-acting analgesic formulations aimed at post-operative treatment have come on the market, primarily for dogs. These are of interest to animal research facilities because they could provide an optimized post-operative analgesic coverage, with less stress due to multiple intramuscular injections. The goal of this study was to compare the effect of a long-acting analgesic preparation with a standard parenteral buprenorphine and NSAID analgesia in minipigs. We compared the postoperative analgesic effect on the GM pigs by analyzing behavioral parameters – interest in surroundings, VAS score, activity, eating behavior, social interaction, movement, posture (tail, legs, head, eyes) and vocalization.

For the behavioral study 29 GM were ovariohysterectomized in general anaesthesia using the Zoletil mixture. Shortly prior to surgery, the GM were given 0.4 mg/kg meloxicam i.m. Postoperatively 15 GM were treated with a standard analgesic regimen of buprenorphine 0.05 mg/kg IM every 8 hours for 24 hours and meloxicam (0.45 mg/kg) given PO every 24 hours for 4 days. Fourteen GM were treated with Fentanyl 1.3 mg/kg post-operatively. The plasma concentrations showed variability, the plasma concentrations were above 0.3 ng/ml after the 3 h time point and up to at least 72 h in two of the three GM, indicating that the chosen dose levels were on the low side. However, there were no significant differences in any of the measured behavioral parameters between the two analgesic groups, indicating that Recuvyra® had a sufficient postoperative analgesic effect in GM in combination with meloxicam.

PC9

Real time rat grimace scale – A method for post-surgical pain control

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Abstract
In experimental surgery, post-surgical pain assessment is still a neglected issue. Pain assessment in rats did not meet proper requirements, but nowadays, Rat Grimace Scale (RGS) provides an accurate and reliable way to quantify pain. For rats, buprenorphine, in addition to other opioids is recommended for alleviation of moderate to severe pain, but few relevant supporting data are available. In the present study we investigate the analgesic potential of buprenorphine in an experimental bone defect in rats. Twelve male, Wistar rats were used; nine were subjected to a non-critical surgically induced bone defect while three were left as reference group. Buprenorphine subcutaneous injections were performed 30 minutes pre-operatory, in two doses of 0.01 mg/kg and 0.03 mg/kg, three animals for each dose, while the remaining three received saline isotonic solution as placebo. Postsurgical analgesic evaluation was done using a real time RGS scoring method for 8 hours long. Placebo animals exhibited moderate pain ranging during observation time between 0.5–0.9. In the analgesia groups, both buprenorphine doses successfully reduced pain (0.09–0.3) starting at 2 hours and up to 8 hours postoperatively. Despite both doses having analgesic potential compared to the control group, a higher effect is conferred by the dose of 0.03 mg/kg (0.09–0.2) throughout the whole experimental period. These results offer unprecedented evidence for the need of analgesia during surgically induced bone defects in rats, highlighting the usefulness of the RGS method for the assessment of animal suffering in surgical experimental procedures.

PC10

Refining anesthesia in mice immune cell in-vivo imaging: Improving animal welfare and internal validity

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Abstract
Anaesthetics can bias experimental readouts undermining internal validity and translation. We aim to quantify the effect on physiology and on the immune system of ketamine/xylazine (KX) with and without O_2, ketamine/xylazine/acepromazine (KXA) and isoflurane (ISO) medium-term (3h) anaesthesia in mice.

N = 24 C57BL/6J mice (m & f; 6-8 weeks) were randomly assigned to ISO (2% + 0,1mg/kg buprenorphine), KX (100/10 mg/kg), KXO_2 (100/10 mg/kg + 100% O_2) and KXA (100/10/3 mg/kg). Popliteal lymph-node exposure was performed and physiological parameters were recorded for 2h.

Re-dosing of half dose followed by one quarter dose every 30' in KX and every 60' in KXA maintained adequate anaesthesia. Oxygen saturation (SpO_2) severely decreased in KX (<70%) and KXA (<80%) compared to ISO (>95%). Additionally, mortality in KX and KXA reached 100%. In KXO_2, SpO_2 remained over 95% and mortality decreased to 20%. The respiratory rate [median and (range)] of KX [155(99-166)], KXO_2 [150(143-155)] and KXA [142(99-152)] was higher than ISO [104(81-131)]. Contrarily, ISO had higher heart rate [482(408-502)] than KX [348(270-444)], KXO_2 [337(295-354)] and KXA [308(240-404)].

O_2 is crucial for mice survival and it must be supplemented even in injectable anaesthesia. Further, we will evaluate the role of the described anaesthetics on the preservation of immune functionality using 2-photon-intravital-imaging. Refinement of anaesthesia will contribute to animal welfare by reducing morbidity and the number of animals necessary to obtain robust results, improving the internal validity of immune cell *in-vivo* imaging.

PC11

Refining experimental strategies to improve the validity of complex trauma models

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Abstract
Modelling traumatic injury remains challenging due to its intrinsic severe nature and complex onset. Trauma is a growing problem worldwide, accounting for 10.1% of the global burden of disease. Cardiac dysfunction (CD) is the principal mode of trauma haemorrhage death after admission. However, its pathophysiology is unknown and therapeutic targets are required.

We report the development of a unique mouse model of trauma haemorrhage induced CD with echocardiographic guidance of fluid resuscitation as a mechanistic approach to investigate the impact of trauma on myocardial function. Mice are subjected to trauma (soft tissue and bone fracture) and haemorrhage and then undergo various echocardiographic-guided resuscitations to restore baseline stroke volume. All studies are carried out under non-recovery anaesthesia with intensive physiological monitoring to simulate ICU settings. At 3h post-injury, these animals have a lower cardiac function (~80% decreased stroke volume), and elevated levels of myocardial injury biomarkers (h-FABP), mirroring the CD seen in trauma patients. Using this modelling platform we have tested the potential role of cardiovascular protective agents to rescue the CD after trauma and showed improved cardiac function, myocardial integrity and overall better haemostasis. These findings are now progressing into further clinical testing in trauma patients.

Our ''From Bench to Bedside and Back Again'' modelling strategy [1] in combination with translational relevant cardiovascular imaging have improved the predictive validity to our animal work, demonstrating the value of using clinically integrated strategies together with robust experimental refinements such as preclinical imaging.

PC12

Not quite the truth: Exploring the published use of the rat grimace scale

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Abstract
Collecting all the published records that report on the use of the rat grimace scale (RGS), almost a decade after its origination, we find that it is a tool unparalleled in its ability to detect pain. Too much so. Even under the ideal conditions of the original proof-of-concept study, the RGS should fail to detect pain in at least one of five studies. This is not the case, however. With meta-analytical methods, it can be demonstrated that the published RGS track record is deeply problematic. Not only can it be suspected that a number of negative findings have been suppressed, but many of the published studies also feature undisclosed data treatment and poor statistics. A majority of the studies have furthermore taken insufficient measures to prevent the researchers' biases from influencing the results.

In preclinical neurobiology – of which pain research is a part – more than 75 % of published findings cannot be reproduced. The outlined investigation into one of the grimace scales used to assess pain in laboratory animals serves as a reminder of the underlying causes. No one effort can turn this around. However, the present investigation highlights elements that can be improved (also outside the use of the RGS): taking measures to eliminate bias, developing a better understanding of experimental design and statistics, employing transparent reporting, and rejecting the rat race for significant findings, proudly publishing our negative/null findings.

PC13

Evaluation of alfaxalone-midazolam anesthesia and flumazenil reversal efficacy in Egyptian fruit bats (Rousettus aegyptiacus)

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Abstract
The Egyptian fruit bat (*Rousettus aegyptiacus*) is a new emerging model in systems neuroscience. Exploration of suitable anesthetic combinations for this species will allow refinement of procedures requiring anesthesia or sedation. Here we characterized anesthesia with alfaxalone-midazolam, as well as the effectiveness of flumazenil, a benzodiazepine antagonist reversing midazolam, in improving recovery duration and quality.

Using a randomized, blinded, crossover trial, ten male Egyptian fruit bats were anesthetized with alfaxalone (15 mg/kg) and midazolam (2 mg/kg), administered subcutaneously. Anesthetic depth and vital signs were monitored every ten minutes. Sixty minutes following anesthetics administration, flumazenil (0.3 mg/kg) or saline were administered subcutaneously. Time to induction, total anesthesia duration, and recovery time were measured. Quality of induction, anesthesia and recovery were assessed on a three-point scale (1 = poor, 3 = excellent).

Induction time was 4.2 ± 1.9 minutes (mean $\pm$ SD), with quality score of 2 (median; range 1–3). Time to first movement was 50 ± 12 minutes, with anesthesia quality score of 3 (1–3). Notably, during anesthesia penis length was significantly increased from baseline, and may be an indicator of anesthetic depth. Administration of flumazenil significantly reduced mean recovery time compared to saline (10 ± 5 versus 45 ± 17 minutes, respectively), and significantly improved the quality of recovery (2.5 [2–3] versus 1 [1–2]).

In summary, alfaxalone-midazolam anesthesia produced smooth induction, good relaxation, and sufficient anesthesia to perform routine diagnostic and therapeutic procedures for approximately 50 minutes. It is recommended to reverse this combination with flumazenil for quicker and better recovery.

PC14

Improving housing conditions ensures health and well-being of animal model: A case of study

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Abstract
Barrier facilities allow to maintain pathogen-free animals, improve animal welfare and ensure experimental data are no longer biased by infection-dependent alterations. In this study we focus on the effects of different housing conditions (conventional *vs.* barrier) on health and well-being of the hyperbilirubinaemic Gunn rat, a unique model for the human Crigler-Najjar syndrome.

The conventional colony established in 2005 was always maintained under conventional conditions, whereas the barrier colony established after rederivation in 2014 was bred under microbiologically controlled conditions (SPF). The two colonies was analysed in terms of reproductive performance and total serum bilirubin (TSB), and data were statistically compared. TSB level in normobilirubinaemic Gunn rat housed in barrier facility did not changed in respect to the levels observed in the same genotype under conventional housing conditions. Notably, a different TSB

concentration was observed in the first week of life in heterozygous pups under conventional housing, presenting a mild hyperbilirubinemia, that disappeared under pathogen-free housing conditions. Moreover, a different trend was also observed in conventional *vs.* barrier housed hyperbilirubinaemic rats. Despite increasing immediately after birth in both colonies, the pick of TSB level was arising earlier under conventional condition, whereas under barrier housing was increasing more gradually and picking later. In the last developmental timing under analysis no statistical difference between the two colonies was observable. The pathogen free housing condition allowed improving the experimental setting, using the heterozygous litters as control for the jaundiced pups, and reducing the need of breeding and usage of additional (wild type) animals.

PC15

Pre-weaning mortality of mice housed in individually ventilated cages with and without controlled humidity

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Abstract
Environmental conditions such as relative humidity (RH) may influence reproductive performance and animal welfare thus affecting research outcomes. Maintaining a stable humidity level in animal facilities is challenging in Finland when RH decreases very often below 40% during winter months. Genetically modified mice were maintained either in humidity controlled (ScanClime) or non-humidity controlled (Gree Line) individually ventilated cages (IVC). We retrieved data from our ELLI record-keeping system related to 246 breeding pairs, 628 litters and 3970 born pups and analysed the pre-weaning mortality from the calendar year 2018. RH recorded in animal room was higher during summer months (51–60%) compared to winter (40–46%). RH in ScanClime system was more stable during winter while the RH in animal room showed seasonal fluctuations. Analysis of the data from the whole calendar year showed a significant reduction in pre-weaning mortality in ScanClime system when compared to GreenLine (16% vs 23%; $p<0.01$). Higher pre-weaning mortality during winter months was observed in both systems. In ScanClime the difference between winter and summer was significant (20% vs 8%; $p<0.01$). However, offspring production during low humidity period (winter) showed that pre-weaning mortality was lower in ScanClime when compared to GreenLine (22% vs 32%; $p<0.05$). In conclusion, our full year and seasonal observations showed that pre-weaning mortality of GM mice strains was significantly lower in humidity controlled IVC system.

PC16

Comparative assessment of stress levels, breeding and welfare of mice in different IVC brands

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Abstract
The aim of this study is to evaluate the welfare, breeding performance and stress levels of C57BL/6OlaHsd mice in four of the most common IVC brands. We evaluated several parameters for breeding cages: number of litters per cage, number of weaned pups in first litter, the total number of weaned pups, weight at weaning and calculated the productive efficiency index (PEI) – number of weaned pups per mother per week. We also evaluated different parameters for "stock" cages: body weight, clinical condition score, and corticosterone levels in urine and in fur. No significant differences were found between the IVC brands in any of the evaluated parameters. The PEI average was 1.36 the weight of the mice at weaning was between 10 grams to 13.86 grams with an average of 11.9 grams. Significant differences were found however, in corticosterone level measured in 6 out of the 8 cages between day 0 and day 30, we believe that the difference between measurements in the same cage is due to lack of acclimation in day 0. Conclusion: None of the parameters measured indicated a significant difference between the four manufacturers. Our conclusion is that mice adapt well to different IVC systems; regarding their general welfare as expressed in breeding performance, clinical condition and levels of stress hormones- after given proper acclimation time. Hence, when choosing an IVC system from these brands additional parameters such as the animal caretaker and the researcher preference, ergonomic factors, the technical service provided, and cost should be considered.

PC17

Is ‘‘BARBERING‘‘ an indicator for mouse housing quality?

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Abstract
Introduction: ''Barbering'' (''fur or whisker trimming'' /''plucking'') – nibbling or plucking off hair – is a widespread phenomenon in laboratory mouse facilities. The question arises if barbering is a suitable indicator for the quality of housing conditions in laboratory mouse husbandry.
Methods: Beside relevant literature analysis barbering was assessed in a long-term C57BL/6 colony and in several mutant lines housed in different experimental barriers.
Results: There are only few relevant studies in the current literature. However, data are difficult to compare due to imprecise definitions of barbering and differing housing conditions. The incidence of barbering is increased in females and in certain mouse strains, especially C57BL/6. Thereby, strain-specific alopecia has to be considered. Furthermore, ''self-barbering'' is also described. Long (1972) supposed barbering as indicator for social hierarchy, whereas Garner (2004) suggested an obsessive-compulsive disorder as possible reason. Only few studies could show a decreased incidence of barbering due to improved housing conditions. Our own investigations suggest that also strain and substrain specific differences has to be considered. In breeding-groups or groups of male mice, barbering might be related to social hierarchy. However, we found groups of older female mice, where almost all animals were affected, indicting mutual fur trimming to strengthen social relationships.
Conclusion: The underlying causes for barbering are still unknown and differing considerations are difficult to reconcile. Barbering as a potential indicator for housing quality is conceivable, but due to the considerable influence of genetic as well as hormonal factors, it cannot be recommended at the moment

PC18

The effect of music upon the behaviour of shelter dogs

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Abstract
There are a lot of previous researches on the effects of listening music for different species, including humans, farm animals, wild animals in captivity and even dogs. Keeping dogs in kennels is a common method in laboratories and rescue shelters. This method may cause stress and behavioral problems is these animals and may decrease their chance of getting adopted.

We studied five mongrel (mixed breed) dogs for twenty days kept in the same kennel. They heard four genres of music for eight hours each: classical, reggie, binaural and for control, white noise. The dogs spent significantly less time in the back and more time in the middle part of the kennel during the classical music and they were more active. We didn't find significant difference between music genres in eating, drinking, tail-wagging, jumping, scratching, barking and interactions, but during classical music the dogs played and wagged their tails more than during any other music.

Finding a type of music or acoustic smituli, which is able to decrease the stress level and is able to modify the behaviour of dogs in a positive way may enchance not only animal welfare in shelters and laboratories but can increase the chance of getting adopted. Furthermore playing music to captive animals can help adapting to excessive noise.

PC19

Effect of noise exposure on the lipidperoxidation in the brain of mice

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Abstract
Noise might be a dangerous environmental stressor. In this study we invastigated the effect of noise treatment upon the free radical and antioxidant levels of the brain tissue.

We used 12-12 male and female Crl:CD1(Icr) mice, and performed 3-minute long open-fiend tests (OF) with them. During the OF trials the mice were subjected to a noise collection (80-90 dB). After the first OF the animals were split into two groups: the noise treated group (N) received a noise treatment every day for 10 hours (70 dB), while the control (C) group was in a quite room. The OF trials have been repeated after 1 and 2 weeks. After the experiment the brains have been removed and three methods were used to assess the lipidperoxidal state of the tissue.

According to our results the 3-week long noise treatment or the noise during the OFs could not induce clinical stress in mice. But the treatment were able to modify the lipidperoxidation of the brain tissue: in the control groups there were less free radicals in the brain of males than in females, but in the females the noise treatment were able to decrease the lipidperoxidation compared to the control females, while there were no difference between the control and noise-treated males. This means that in female mice the noise habituation might be able to decrease the negative effect of abrubt noises, but in males it is not that obvious.

PC20

Managing singly housed male mice

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Abstract
Directive 2010/63/EU recommendations suggest social housing before and after weaning in mice. However, different situations may lead to male mice isolation since they cannot be regrouped with other male mice due to aggressiveness. There is evidence that individually housed mice displayed alterations of behaviour and global epigenetic changes in the brain. During the last three years, we have implemented two strategies to reduce the number of isolated male mice. The ''companion mouse'' strategy is applied when sacrifices are requested, if only one mouse of a cage will remain alive, we keep one of his established social group mate with him, identified as ''companion'', and both of them will live together. We also group males, which at weaning had been left

alone. The results have shown a progressive reduction in the number of animal welfare incidences due to individualization, registering 42% less isolated male mice in 2018 compared to 2016. In addition, we managed to reduce the percentage of individuals to a value below 10% in all the strains, reaching in instances a percentage below 5%. Interestingly, this reduction was not accompanied with increase in fights. In conclusion, both strategies are suitable husbandry practices to reduce the number of single housed male mice.

PC21

Social enrichment by partner-housing of male C57BL/6JRj mice?

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Abstract
It is a common code of practice to keep laboratory mice in groups. Due to aggression or experimental conditions, males are often housed individually, though social isolation causes welfare issues and differences in behavior.

To provide social enrichment for individually housed mice, we separated 2 male mice for 8 weeks in cages divided by transparent, perforated walls allowing sensory contact. The effects of partner-housing on behavior and stress hormones were compared with data obtained from single- and group-housed mice. Besides burrowing and nesting, we assessed anxiety-related behavior in the free exploratory paradigm, ease of handling by using a rating scale and social behavior in a social interaction test.

No significant differences were detected in burrowing between the groups. Partner-housed mice built nests of a higher complexity when compared to single- and group-housed mice. Moreover, it took partner-housed mice longer to contact experimenter's hand than single-housed individuals. Group-housed mice showed a reduced flight reaction compared to single- or partner-housed mice. The free exploratory paradigm did not reveal any difference in the latency to explore, indicating no effect on trait anxiety. Only single-housed mice showed significant preferences for an unknown mouse in the social interaction paradigm. However, partner-housed mice moved significantly less in the test arena.

Preliminary results showed no conclusive differences in behavior. The decrease in activity and increase in latency to hand contact suggested elevated state anxiety, while nesting and burrowing indicated general well-being in partner-housed mice. Levels of stress-hormones will be analyzed but are not available yet.

PC22

Castration of adult male mice allows resocialization and social housing of previously single-housed males

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Abstract
Due to aggressive behavior among male mice, it is common to house them individually. However, social isolation causes serious welfare problems. In a case-study approach, we seek to demonstrate that castration of males, which had been individually housed for a longer period because of interspecific aggression, allows resocialization and harmonious group housing. Mice of 2 strains, BALB/cJRj mice (n = 21) and C57BL/6JRj mice (n = 19), were castrated. Following castration, the Mouse Grimace Scale, burrowing as well as nesting, weight progression and fecal corticosterone metabolite excretion were evaluated. 4.5 weeks after castration, the mice were resocialized in groups of 3–4 animals and indicators for group stability, i.e. interaction, group nest scores, and body weight, were investigated. In mice of both strains, neither burrowing nor nesting was impaired on day 1 after castration. In C57BL/6JRj mice, pain faces were found for up to 150 min post-surgery; stress hormone metabolite excretion increased on day 2 after castration and decreased by day 14 when compared to baseline. When animals were resocialized, C57BL/6JRj mice showed no aggressive behaviour. Aggressive behavior of the BALB/cJRj mice was limited to the first hours of group housing. Mice of both strains lost weight over the first two weeks and then regained weight. Our investigations demonstrate that castration of adult male mice allows group housing. In terms of a lifetime harm-benefit analysis for the individual animal, the short-term post-surgical stress and pain may be preferable to the long-lasting negative effects of single-sex housing of uncastrated males.

PC23

Reducing aggression in Cast/Ei-mice

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Abstract
Mice of the strain *Mus musculus castaneus* (Cast/Ei) were derived from wild mice.

As they are genetically distinct from common laboratory mice, they are valuable models for genetic mapping, evolution and systematics research. Under captive conditions, Cast/Ei-mice show

signs of stress, ethopathies, and aggression against cage mates. Our study focuses on two ways to reduce this behavior. Cast/Ei-mice will either get an energy-reduced diet or an igloo with running plate. Control mice will be housed with standard diet and enrichment. Giving the mice the possibility to run or to spend more time foraging and digesting, simulates their natural behavior, which should reduce the burden of captivity. These refined housing conditions can improve the welfare of Cast/Ei-mice in research.

PC24

Navigating the Vortex of Rodent Importation

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Abstract

1. Develop a comprehensive Rodent Import Request Form to identify the appropriate contact.
2. Review your institution's health status and develop criteria for rejection.
3. Ask questions.
4. Ask the questions again in a different way.
5. Quarantine – Count the ways you can protect the health status of your institution.
6. Provide options for denied requests.

Importation of rodents is an intimidating endeavor. Establish a request form that includes essential information regarding the exporting institution's health status and husbandry practices. Verify that the information is correct. Determine rules, if your institution operates as an SPF facility (ies). A simple rule is do not accept animals from institutions with a history of pathogens or parasites within the past 12 months. Review health/sentinel testing results and be suspicious if all testing is ''in house'' with no reference laboratory values. If received test results are in a spreadsheet after laboratory testing, ask to see the laboratory results. Review health-monitoring results for the entire institution, or at least all facilities that share a cage wash facility and/or husbandry staff. If test results are negative, ask for a description of historical outbreaks and the eradication methods utilized. This often reveals a pattern of disease that received ineffective treatment. Many viruses and parasites are hard to detect, so be wary of negative test results. If received into quarantine, fecal PCR testing at days 0, 7 and 14 post-shipping provides assurance. Lastly, rapid rederivation offers to many an affordable option

PC25

Mouse preputial gland abscesses in a GM breeding colony: Prevalence and effective measures

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Abstract

During one year observation in our mouse barrier breeding unit, a high incidence of preputial gland abscesses (PGA) was detected i.e. between 19-38 affected male mice every month. This raised both animal welfare and scientific concerns. The suppurative exudate and fecal material obtained from a representative number of cases were submitted for microbiological analysis. *Staphylococcus aureus* (*S. aureus*) was the only isolated bacteria from the exudate. In the fecal material we found *S. aureus* (79%), *S. xylosus* (5%) and a combination of both (16%). Because of the opportunistic pathogenic nature of these bacteria, we investigated their presence in the feces of healthy mice. Screening for *S. aureus* and *S. xylosus* was extended to all genetic modified (GM) strains present in the unit. We found that 22% of the strains (44/201) were positive for *S. aureus* and 73% (147/201) were positive for *S. xylosus*. Interestingly we found that 9 GM strains although positive for *Staphylococci* did not develop abscesses in previous years. This shows the presence *Staphylococci* alone was not sufficient to cause PGA. Therefore, we took into consideration other factors such as genetic make-up, age, environment and management related aspects. We concluded that increase in incidence was not linked to a particular genetic background i.e. higher prevalence was not present in the immunodeficient strains, or related to age. Tight clinical monitoring resulting in early detection of growing abscesses, immediate euthanasia of affected animals and stricter cage-changing procedures resulted in a significant decrease of PGA incidences (average 3 cases per month).

PC26

IL-32γ reduces cerebral ischemia reperfusion injury in MCAO mouse model

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Abstract

Ischemic stroke is a major cause of death and the most frequent cause of permanent disability in human health and disease. Cerebral ischemia initiates the pathophysiological pathways of the ischemic cascade and eventually causes irreversible tissue injury in the ischemic core within minutes of the onset. Post-ischemic inflammation has been considered as a major contributor to ischemic neuronal injury and targeting proinflammatory

cytokines such as IL-1β, TNF and IL-6, are of great interest in patients with stroke. IL-32γ, which is a recently described inflammatory cytokine, has been associated with the induction of inflammatory response in various cell types by secreting IL-1β, TNF and IL-6. In the present study, we demonstrated the role of IL-32γ in surgically induced MCAO model using a transgenic (TG) mice expressing human IL-32γ. While comparing with WT mice, IL-32γ TG mice exhibited a significantly reduced infarct area following MCAO surgery. Associated with attenuated brain tissue damage, levels of IL-1β, TNF and IL-6 were significantly reduced in the IL-32γ TG mice compared with WT mice. These results suggest that IL-32γ can prevent cerebral ischemia damage and improve neurologic impairment symptoms.

PC27

Analysis of background data in young C57BL/10Sc-mdx mice

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Abstract
CIEA maintains a variety of muscular dystrophy model mouse strains and has supplies them to several research institutions. The *mdx* mouse are the most common mouse model for Duchenne muscular dystrophy (DMD). To contribute to muscular dystrophy research, we analyzed the body weight, organ weight, clinical biochemical, and historical background data of young C57BL/10Sc (B10)-*mdx* (B10-*mdx*) mice and compared these to the data obtained from B10 mice (10 animals/group/sex, 3–10 weeks of age). We showed that the brain weights of B10-*mdx* mice were lower than that of B10 mice, and enlarged lateral ventricles were obviously detected in B10-*mdx* mice. However, no clear histological differences were observed between the brains of the B10-*mdx* and B10 mice. Serum/plasma CpK, LDH, AST, and ALT levels in B10-*mdx* mice significantly increased from 3 weeks old when compared to B10 mice. At 5–6 weeks old, CpK levels in B10-*mdx* mice were especially high. Histopathology of the skeletal muscle in B10-*mdx* mice revealed dystrophic changes with active muscle fiber necrosis and regeneration. From 6 weeks old, fibrosis in the hindlimb muscles was higher. These results show that B10-*mdx* mouse have similar phenotypes to human DMD. Therefore, the *mdx* mouse model is an extremely useful animal model for DMD.

PC28

Effect of cetrorelix acetate administration on ovarian stimulation in aged mice

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Abstract
In mice, ovarian stimulation by administering a hormone is an effective method to obtain many ova synchronously, but its effect is reduced by the influence of aging. In this study, we demonstrate that this problem can be improved by administering the gonadotropin-releasing hormone antagonist cetrorelix acetate (Cetrotide, Nippon Kayaku Co., Ltd.) prior to ovarian stimulation. Before 12-month-old female mice were injected with 5 IU pregnant mare serum gonadotropin (Serotropin, ASKA Pharmaceutical Co., Ltd.) and 5 IU human chorionic gonadotropin (Gonatropin, ASKA Pharmaceutical Co., Ltd.), we administered 5 μg/kg cetrorelix acetate for 7 consecutive days or 3 times once every 3 days. As a result, 9 ± 2 (mean ± standard error of the mean, n = 10) and 10 ± 1 (n = 10) oocytes were obtained, respectively, as opposed to 5 ± 1 oocytes (n = 9) in the case of no administration. Collagen staining of ovarian tissue showed that cetrorelix acetate administration reduced the degree of fibrosis, which resulted in the improvement of ovary function. In addition, fertilization and fetal development rates equivalent to those of young mouse-derived oocytes were confirmed by *in vitro* fertilization and embryo transfer (88.5/96.9% vs. 92.2% and 51.8/58.3% vs. 56.4%, respectively), indicating the normality of the obtained oocytes. This procedure will contribute to animal welfare by the effective and extended utilization of aged female breeding mice.

PC29

Role of neuropeptide Y in maternal behavior of the female native Thai chicken

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Abstract
Neuropeptide Y (NPY) involved in food intake regulation in birds and mammals. Changes in food intake are noticeable during their reproductive stages in which incubating hens eat and drink less when compared with rearing hens. To investigate the role of NPY during the transition from incubating to rearing behavior in the native Thai chickens, the distributions of NPY-immunoreactive (-ir) neurons and fibers within the paraventricularis magnocellularis nucleus (PVN) were compared between incubating hens (INC) and hens for which the incubated eggs were replaced with 3 newly hatched chicks for 3 days after 6, 10, and 14 days of incubation (REC). Using an immunohistochemistry technique, the results revealed that the number of NPY-ir neurons in the PVN decreased

in the REC13 hens when compared with the INC13 hens (INC13 vs REC13; 5.20 ± 1.52 vs 2.30 ± 1.20 cells; $P < 0.05$). However, changes in the number of NPY-ir neurons between the INC and REC hens at days 9 and 17 were not observed. Apparently, the increased distributions of NPY-ir fibers in the REC hens when compared with those of the INC hens were noticeable at all 3 different time points. These present results demonstrate that feeding behavior affects the distribution of NPY neurons in the PVN during the transition from incubating to rearing behavior, confirming the pivotal role of the NPYergic system in the regulation of feeding and maternal behaviors in avian species. In addition, native Thai chicken might be an excellent animal model for the study of this phenomenon.

PC30

Mancozeb impaired male fertility in rabbits with trials of glutathione detoxification

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Abstract
The study aims to evaluate the potential reproductive toxicity induced by mancozeb fungicide in male rabbits, and to examine the ameliorative effect of glutathione (GSH), a non-enzymatic antioxidant, against mancozeb reproductive toxicity. Mancozeb is a member of the dithiocarbamates group currently in use in the management of fungal diseases of plants. It was addressed for male reproductive toxicity.

To achieve these aims, mature male White New-Zealand rabbit of 4-5 month old were randomly assigned to four groups of 9 animals each: control, mancozeb only, mancozeb and GSH, GSH only.

This study discovered a significant reduction in serum FSH, LH, testosterone and testicular LDH, ACP, and ALP levels in a group of mancozeb treated rabbits compared with control. The mancozeb only group also showed a significant loss in sperm viability along with a significant increase in the numbers of abnormal sperms were realized. Finally an upper regulation in steroidogenic 3β-HSD enzyme activity was noted in mancozeb only treated rabbits. Histopathological inspection of testicle established disruption of germinal epithelium with vacuolization of Leydig cells and reduced spermatogenic cells.

GSH co-administration increased serum concentrations of FSH, LH, testosterone, and of testicular enzymes levels: LDH, ACP, and ALP. Improved steroidogenesis, was indicated in this group by significant improvement in testicular 3β-HSD enzyme, by significant increase in sperm viability, and by a significant decrease number of abnormal sperms.

The findings of this study suggested that mancozeb exposure has antispermatogenic and anti-steroidogenic adverse effects in rabbits and administration of GSH may alleviate the reproductive toxicity.

PC31

Superovulation in Xenopus tropicalis – Protocol refinements

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Abstract
Superovulation is a common technique to gain high numbers of oocytes from individual females at a defined time point. A potential suffering of the animals has to be balanced against the reduction of animal numbers. The technique itself should be compatible with the general well-being of the animals. Superovulation is successfully used in Xenopus laevis and allows repetitive treatments. We obtained a presumably well established protocol for the superovulation of Xenopus tropicalis from a collaborating laboratory. However severe health problems occurred in a high number of the frogs. After application of 20 IU PMSG and 200 IU hCG 24h by i.m. or s.c. injection, we observed severe sickness and sudden death in ten out of 40 female Xenopus tropicalis within 17 days post treatment. Gross pathology and histology indicates an ovarian hyperstimulation syndrome with substantial subcutaneous edema and massive effusion in the body cavity. In the next step, we tested a combination of two hCG treatments. For the second injection, between 60 to 200 IU hCG were applied. With this approach, we identified the combination of 10 IU hCG at day 1 and 90 to 130 IU hCG at day 2 as highly effective by means of oocyte amount and quality in combination with an obvious improvement of animal welfare. Besides the hormone dosages, we identified the injection as a key procedure. Due to the very small size of the frogs, it was necessary to explore the most careful procedure of fixation of the animals.

PC32

Identification of potential endocrine disruptors using alternative methods according to 3R principles

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Abstract
Endocrine disrupting substances comprise various chemicals (e.g. cyclic hydrocarbons, phenols, flavonoids, phthalates, parabens, biocides, plasticizers, surfactants, fire retardants, antimicrobials, UV filters). Hormonally active compounds are found in environmental and biological samples, consumer products, food, plastics, food contact materials, etc. *In silico* (QSAR) and *in vitro* screening methods (test according to OECD TG 455 using cell line VM7Luc4E2 and yeast-based microplate assay XenoScreen, supplied by Xenometrix, CH) were used for identification of endocrine

disruption potential of seven analogues of bisphenol A and seven phthalates. *In silico* results obtained with the use of OECD QSAR Toolbox predicted all the screened phthalates as non-binders and all the screened bisphenols as very strong binders on estrogen receptor . A scientifically valid QSAR Toolbox for the androgen receptor is not available yet. Both *in vitro* biological methods exhibited good concordance of results regarding the estrogenic agonistic activity considering minor discrepances due to cytotoxicity elicited in higher concentrations. Substances showing negative estrogenic activity may exhibit activity on the androgen receptor. New chemicals being developed as replacement of compounds already regulated as endocrine disruptors (e.g. BPA) should be a subject of thorough evaluation to avoid their contribution to adverse health effects caused by exposure from multiple sources. Alternative methods based on human cells and tissues are promising tools for identification of endocrine disruption in terms of systemic toxicity. The research was supported by ERDF/ESF project "International competitiveness of NIPH in research, development and education in alternative toxicological methods" (No. CZ.02.1.01/0.0/0.0/16_019/0000860).

PC33

Tamoxifen affects body weight and seminal vesicles in mice but does not induce hernia

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Abstract
Introduction: Ma et al., 1852(5), report severe hernia in 10 week old C57BL/6-males after feeding a diet with 20mg Tamoxifen (TAM)/kg, lasting five weeks, with unknown genetic background. Others report TAM having an impact on the body weight and the seminal vesicles. We monitored for hernia formation in mice which were fed TAM to induce CRE-activation.
Methods: Male mice (n = 10) (C57BL/6N and C57BL/6J) received a TAM-containing diet (240mg/kg chow) from the age of 8 weeks for a period of 21 weeks. Another group of older mice (n = 12) received TAM from the age of 21 weeks for a period of 17 weeks.
Results: The induction of the TAM-containing diet led to initial weight loss; later the body weight stabilized and afterwards increased only moderately. The seminal vesicles of all males on a TAM diet were considerably smaller than in the control group. No hernia was detected during dissection. The abdominal wall was uninjured, no organs were protruded extra-abdominally.
Conclusion: The influence of TAM on the body weight and the seminal vesicles could be confirmed. No hernia occurred, despite the 12x higher TAM-concentrations compared to Ma et al., and despite the 3-4x longer feeding period. This discrepancy could be caused by differences in the genetic background, the dosage and duration of feeding TAM, or the form of administration. TAM is frequently used in combination with the CRE/loxP system; in these experiments it is thus important to be aware of the various side-effects.

PC34

Refinement of the adenine model for chronic kidney disease in rats

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Abstract
The chronic kidney disease (CKD) models in rodents are associated with high disease burden and strong suffering for the used animals. Our aim is to develop a more practical, convenient and accurate CKD model with less suffering for the animals and used a previously published adenine injection model with 6-month-old male WISTAR rats (Al Záabi, M. et al. 2015). In the first experiment, we used two amounts of adenine 50 mg and 100 mg adenine per kg bodyweight as published. Surprisingly, none of the rats survived the experimental period of 56 days. Therefore, we did several titration studies to find a sufficient adenine concentration to induce a CKD without inducing human endpoint criteria.

In our final setup, we defined an amount of 35 to 40 mg adenine per kg bodyweight administered once daily for 28 days to induce a moderate CKD in adult WISTAR rats. The CKD model was associated with significant changes in: the histological sections of the kidneys, increase in plasma phosphate and creatinine, a decrease in plasma calcium, a decrease in creatinine and phosphate in urine, a decrease in creatinine clearance, and a decrease of around 6% in body weight. None of the animals died or reached the humane endpoints during the experiment. This refinement of the CKD model in adult WISTAR rats results in a reproducible and stable model with a reduced burden for the used animals.

PC35

IgA nephropathy benefits from LCHK168 therapy by enhancing SIRT3/autophagy-mediated NLRP3 inflammasome inhibition

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Abstract
IgA nephropathy (IgAN), the most common primary glomerulonephritis, follows a relatively poor prognosis yet lacks a pathogenesis-based treatment. LCHK168 is a major absorbable intestinal bacterial metabolite of ginsenosides, the latter being bioactive components from ginseng. Herein, we examined whether the therapeutic effect of LCHK168 on IgAN in mice. In light of the potential anti-inflammatory action of ginseng-derived pure compound LCHK168, we verified its therapeutic effects on two complementary mouse IgAN models – a passively induced IgAN model provoked by repeated injections of IgA immune complexes and the other a spontaneous grouped ddY mouse model. In addition, we determined whether LCHK168 could modulate the axis of sirutuin 3 (SIRT3) and autophagy expression, thereby inhibiting the activation of NLRP3 inflammasome. The present study first revealed that ginseng-derived pure compound LCHK168 ameliorated glomerular mesangial cell proliferation, leukocyte infiltration, sclerosis, and renal tubulointerstitial inflammation in a passively induced or spontaneously occurring IgAN model. The beneficial effects of LCHK168 were in connection with: [1] inhibition of NLRP3 inflammasome in renal tissues, macrophages and dendritic cells; [2] enhanced expression of autophagy in renal tissues, macrophages and mesangial cells; and [3] enhancement of SIRT3 expression in macrophages and mesangial cells. Thus, LCHK168 treatment markedly improved the renal condition associated with enhancing the axis of SIRT3/autophagy-mediated NLRP3 inflammasome inhibition as a major mechanism of action for the ginsenoside-derived pure compound LCHK168 on both the IgAN models. Our results support LCHK168 as a potent drug candidate for IgAN.

PC36

Voltage-gated sodium channels inhibitors as potential strategy for therapy of pathological excessive cough

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Abstract
Pathological excessive cough is a serious clinical problem in many patients regarded to be secondary to inappropriate activation of vagal sensory nerves. Respiratory nodose Aδ-fibres and jugular C-fibres are involved in regulation of cough. Current advances in understanding of voltage-gated sodium channels (NaVs) lead to the rational hypothesis that drugs capable of selective blockade of NaVs subtypes may be an effective and save strategy for the treatment of pathological cough in comparison of centrally acting antitussives with undesirable side effects. Recent electrophysiological studies revealed that NaV 1.8 plays a key role for action potential initiation in C-fibres. We aimed to evaluate the effect of locally applied (inhaled) selective NaV1.8 inhibitor (A-803467,3 mM) on cough in guinea pigs tussive challenge model. We used a standard TRPV1 receptor activator capsaicin (25 μM) to evoke cough. An experimental group was pretreated with NaV1.8 inhibitor by inhalation of aerosol (A-803467,3 mM) for 10 min followed by inhalation of capsaicin aerosol together with inhibitor for 5min. No analgesic treatment was required and no respiratory distress was observed. In control experiments, we observed a reproducibility of cough response to inhalation of capsaicin (5.73 ± 0.6 vs. 5.8 ± 0.35, $n = 15$). Preinhalation and continuing inhalation of NaV1.8 inhibitor A-803467 blocked capsaicin-induced cough (5 ± 0.47 vs. 1.9 ± 0.35, $n = 13$, $P < 0.01$). A similar response was observed in electrophysiological studies where the bradykinin-induced action potential discharge in jugular C-fibres was by 50% inhibited by NaV1.8 blocker.
Conclusion: Our results support a concept that targeting NaV1.7 and NaV1.8 is a rational strategy forward for the effective treatment of pathological cough.
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PC37

Evaluation of using different mouse strains for Ebola virus isolation

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Abstract
During the Ebola outbreak in 2014-2016 in West Africa several reoccurrence of Ebola virus disease (EVD) cases were reported due to long-term virus persistence in diverse body fluids. To estimate and mitigate the risk of transmission from survivors, nucleic acid based methods are not suitable diagnostic tools to provide appropriate information about contagiousness of the body fluids. To evaluate the most sensitive virus isolation animal model for the West-African Ebola virus strain suckling mice (BALB/C and C57BL/6 populations) and SCID mice were used. Virus isolation was attempted from diverse clinical samples (eg. urine, whole blood, semen). The clear humane endpoint was defined as a 20% body weight loss, for pain management non-pharmacological pain alleviation (such as soft bedding) was set up. For in vitro isolation VeroE6 cells were used. All work with infectious agents was performed under biosafety level 4 (BSL-4) conditions at National Biosafety Laboratory, Hungary. Our results highlight that the SCID mice with 30% isolation rate is a sensitive animal model for Ebola virus isolation even with low viral load, however it may take up to 60 days for the symptoms to develop. The suckling mice model showed elevated sensitivity when compared to VeroE6 cells. Moreover, for monitoring the viremia in SCID mice, the non-invasive saliva sampling method was proven to be suitable alternative over the tail-vein blood. Hence, SCID mice could be a sensitive

animal model for Ebola virus isolation to obtain reliable data on the infectiveness of the body fluids of sick patients and survivors.

PC38

Rat TNBS-induced colitis: Refining an old model with new tricks

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Abstract
Introduction: TNBS (2,4,6-trinitrobenzene sulfonic acid) is the most popular chemically-induced model of inflammatory bowel disease in rats, but its variability and associated discomfort remain a challenge.
Aim: To compare 3 refinement protocols (RP) of rat TNBS-induced colitis.
Animals and Methods: Protocols were approved by institutional animal welfare bodies. On day 0 (d0) colitis was induced in 8-12 weeks old male Wistar rats (rectal instillation of a TNBS ethanolic solution) after a 24h fasting (*ad libitum* access to a sugary solution). Littermates were used as controls. RP1 (n=17): analgesia was provided by tramadol (d1, 20mg/kg, SC) and paracetamol (d0-7, 6mg/ml, drinking water); RP2 (n=19), the same as RP1, but tramadol was administered on d0 and metoclopramide (d1, 1mg/Kg, SC) was added to avoid intestinal stasis; RP3 (n=19), paracetamol was given with honey (d0-7, 500mg/kg, PO) and metoclopramide was administered on d1. Body weight, food and water intake, fecal excretion, general welfare and grimace scores were daily registered. On d7-10 animals were euthanized and the colon macroscopically scored (MaS) to categorize disease severity as Mild (MaS = 0-4), Moderate (MaS = 0-8) or Severe (MaS = 8-12).
Results: The percentage of Mild, Moderate and Severe animals was similar with RP1 (29.4%, 35.3%, 35.3%, respectively, $p > 0.05$) and RP2 (36.8%, 26.3%, 36.8%, respectively, $p > 0.05$). With RP3, the percentage of Severe animals was lower than that of Mild and Moderate animals (10.5%, 31.6%, 57.9%, respectively, $p < 0.05$).
Conclusion: Improving analgesia and intestinal motility in TNBS-induced rats represents a refinement and improves animal's outcomes.
Acknowledgments: GEDII-Pfizer for funding.

PC39

Identification of a new modifier in a mouse model of inflammatory bowel disease

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Abstract
The etiology of inflammatory bowel disease (IBD) is highly complex and various genetic, environmental and microbial factors contribute to disease development. A very suitable model for IBD is the Interleukin-10-deficient-(*Il10*$^{-/-}$)-mouse. While B6.129P2/JZtm-*Il10*^{tm1Cgn} (B6*Il10*$^{-/-}$) mice are partially resistant to colitis, mice carrying the *Cdcs1* haplotype on chromosome3 (MMU3) derived from susceptible C3H/HeJBir-*Il10*^{tm1Cgn} mice like B6.Cg-*Il10*^{tm1Cgn}MMU3(D3Mit11-D3Mit348)/JZtm (BCR3*Il10*$^{-/-}$) or B6.Cg-*Il10*^{tm1Cgn}MMU3(D3Mit49-D3Mit348)/JZtm (BC-R2*Il10*$^{-/-}$) develop severe disease. BC-R3*Il10*$^{-/-}$ animals compared to BC-R2*Il10*$^{-/-}$ mice have a larger congenic element on MMU3, enabling the analysis of phenotype contributing sub-congenic-elements within the *Cdcs1* interval. The aim of the study was to identify and to confirm potential candidate genes within *Cdcs1*, especially their impact on the colitogenic potential of T-cells. The strains B6*Il10*$^{-/-}$, BC-R3*Il10*$^{-/-}$ and BC-R2*Il10*$^{-/-}$ were genotyped with an array consisting of 77,000 SNPs to confirm the congenic fragment and the B6/J background. Adoptive T-cell transfer into B6*Rag1*$^{-/-}$ revealed that naïve T-cells isolated from BC-R3*Il10*$^{-/-}$ mice induced a stronger phenotype in recipients compared to T-cells isolated from BC-R2*Il10*$^{-/-}$ animals, pointing to a modifier locus within *Cdcs1* that had not been confirmed so far. This newly identified subcongenic element was provisionally named *Cdcs1.4*. Subsequent microarray analysis revealed 9genes that were differently regulated between congenic mice. The expression differences of interferon-inducing-gene-44 (*Ifi44*) located within *Cdcs1.4* were further confirmed by qPCR and Western Blot analyses. In summary, a new subcongenic region, provisionally named *Cdcs1.4* was identified containing *Ifi44* as an important candidate gene for colitis modulation.

PC40

Functional and histological recovery improvement of the experimentally transected sciatic nerve in rats

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Abstract

Introduction Platelet-rich plasma (PRP) products and mesenchymal stem cells (MSC) have an important potential as a neuroprotective and neurogenic therapeutic modulator system.The aim of this study was to investigate if PRP and MSC could enhance nerve regeneration when applied locally in primary repair of peripheral nerve transection, in an experimental rat model.

Materials and methods: Forty-two male Wistar rats 2-months-old were divided into three groups (Control, PRP, and MSC). All subjects were operated under general anesthesia with 0.5 mg/kg of medetomidine and 50 mg/kg of ketamine i.m. on the right leg's sciatic nerve microscopically. The surgical site was injected with normal saline, PRP or MSC (derived from their femoral marrow) in the respective groups. Analgesia with paracetamol was administered to all rats (1 mg/kgr/12 hours for 7 days). Three months post-surgery, electromyography of both hind limbs was performed under general anesthesia, following which the animals were euthanized for histology.

Results: PRP had a statistically significant positive effect ($p < 0.05$) on sciatic nerve potential versus the Control group, while MSC had a positive non-significant effect. Histologically, the neural axons were statistically significantly less reduced in the PRP ($p < 0.05$) and the MSC ($p < 0.05$) groups versus the Control group.

Conclusion: PRP plays a significant role as a neuroprotective and neurogenic healing agent for surgeons to use. MSC also appear to be promising healing agents according to the histology results of this experimental model.

PC41

Plant extract administration and mild daily exercise increase bone density of ovariectomized rats

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Abstract

Background: The ovariectomized (OVX) rat model of postmenopausal osteoporosis is widely used to evaluate therapies that reduce bone loss[1]. This study investigated the effect of the *Ebenus cretica* plant extract consumption, mild daily *ad libitum* exercise[2] and their combination on bone density of this model.

Materials and methods: Forty mature Wistar rats were OVX and randomly assigned into groups of 10 rats. The OVX and *Ebenus cretica* (EC) Groups were housed in standard cages (18 cm height), the Exercise (EX) and EC+EX Groups in two-storey cages (40 cm height). The EC and EC+EX Groups received EC in their drinking bottles. All rats were scanned at the whole tibia and the proximal tibial metaphysis for bone density pre-, 3 and 6 months post-OVX by dual-energy X-ray absorptiometry.

Results: Total tibial bone density median percentage changes from baseline of the groups were: OVX -9.27% and -17.93%; EC -0.44% and -3.67%; EX 0.00% and -1.83%; EC+EX -2.23% and −3.88%, at 3 and 6 months respectively. Proximal tibia percentage changes were: OVX -19.37% and -30.93%; EC -13.90% and -14.74%; EX -12.78% and -21.83%; EC+EX −10.20% and −14.14%, at 3 and 6 months respectively. Statistically significant differences were shown between OVX compared to all other groups regarding total tibia at 3 and 6 months, and proximal tibia at 6 months.

Conclusions: *Ebenus cretica* administration alone, mild daily exercise alone, as well as their combination exerted a statistically significant beneficial effect on bone density loss caused by ovariectomy.

PC42

Refinement of animal welfare in a rat model of arthritis and inflammatory pain

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Abstract

The present study tested the effect of buprenorphine analgesia in an adjuvant induced monoarthritic rat model, with the aim of refining the model with respect to animal welfare.

The study examined effects of buprenorphine administered subcutaneously and by ingestion of chocolate- hazelnut spread in comparison with vehicle and subcutaneous carprofen in rats the first three days after injection of Complete Freund's adjuvant (CFA) in the tibio-tarsal joint of male and female Sprague Dawley rats.

A higher pain tolerance to mechanical stimuli was occasionally observed by Electronic Von Frey testing in the analgesic treatment groups compared to the CFA vehicle group. Seven hours after induction, a significant difference in pain threshold was evident in all buprenorphine groups compared to the CFA vehicle group of both genders. No significant difference was observed between the buprenorphine groups. Joint circumference was highest 24–72 h after CFA injection and all buprenorphine groups were similar to the vehicle group, while reduced in the carprofen group after 48–72 h.

The study demonstrates that buprenorphine in all mentioned formulations has an anti-nociceptive effect in the induction of the adjuvant induced monoarthritic rat model, without obviously compromising the development of the inflammation in both genders. The study also supports previous findings that buprenorphine administered for voluntary ingestion in chocolate-hazelnut spread is a functioning alternative to repeated injections of the drug.

PC43: Withdrawn

PC44

Doses optimization for lipopolysaccharide induced inflammation to minimize pain and distress in mice

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Abstract
Lipopolysaccharide (LPS) is often given intraperitoneally (i.p.) to induce systemic inflammation in models. The effects of different doses of LPS in mice on inflammation parameter such as body temperature (BT) and motor activity (MA) were recorded. LPS doses inducing a moderate and reversible sickness should identifyed. In C57BL6/J mice transmitter were implanted. Basis data of BT and MA was telemetric monitored over several days. Thereafter, i.p. injections of LPS (2mg/kg, 250 μg/kg and 100 μg/kg, n = 5 mices each) were given and BT and MA data were registered in 5 minute intervals for the following days. Directly after i.p. injection of 2 mg/kg LPS the BT decreased (minimum = 31.7°C after 3 h) and re-increase 9h later. Normal BT was reached around 24 h after LPS injection. However, the normal day-night rhythm of BT and MA was reached after two days only. The application of lower doses induced a decrease of MA and an increase of BT for 9 h (250 μg/kg LPS) and 7 h (100 μg/kg LPS), respectively. Normal rhythmic of both parameters was lost for about 24 hours. Whereas LPS given in low doses induced an increase of BT, the injection of higher doses was followed by BT decrease. Daily rhythmic pattern are destroyed and needs different time for restoration. The use of a low dose LPS induced inflammation model can prevent pain and distress from the animal. The information value of such model has to be verified yet.

PC45

Study to establish an optimal model for investigation of diabetesinduced aortic valve disease

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Abstract
Introduction: It is known that the presence of diabetes accelerates calcific aortic valve disease in human patients, but there are few mouse experimental models to study the early molecular modifications and possible preventive therapies.

Because the current models require at least 8 weeks of hyperlipidemic diet, we run a pilot study to investigate whether streptozotocin (STZ)-induced diabetes in mice fed with a hyperlipidemic diet would help the scientific purposes without affecting animal welfare.
Materials and method: Mature ApoE-deficient mice were injected for five consecutive days with low-doses of STZ intraperitoneally. Up to the 8 weeks of study, the mice were evaluated daily for glycemia, weight, food and water intake, and signs of distress. Where appropriate, subcutaneous insulin and fluid support therapy were administered. At given time points, the animals were sacrificed and samples were taken for further histological analysis.
Results: Diabetes onset (>300 mg/dL glycemia) and relevant histostructural changes were detected as early as the first week after last STZ dose. With one exception, all mice maintained a good general appearance thought the experiment.
Conclusions: Our study established an early model for aortic valve disease in diabetic ApoEdeficient mice, which allowed us to use in further experiments a reduced number of animals, for shorter periods of time without additional insulin or fluid therapy.

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PC46

Jerusalem artichoke containing crackers as a possible remedy for obese rats

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Abstract
Obesity is a leading metabolic disease worldwide and represents major risk factors for other diseases that could lead to death. Chitosan and barely meal, but not Jerusalem artichoke, have been used extensively in treating obesity. We aimed at production and evaluation of artichoke containing crackers for possible treatment of obesity. 104 female albino rats were randomly divided into 13 groups (8 rats each). The first group acted as negative control while remaining ones were fed high fat diet (40 %) to act as obese rats. Obese rats in groups 3-6 were fed on jelly containing zero, 2%, 4% and 6% chitosan respectively. Obese rats in group 7-10 were fed on wheat crackers containing zero, 20%, 35% and 50% of artichoke flour respectively. Groups 11-13 were fed on crackers containing 20%, 35% and 50% barley meal respectively. Obese rats fed on high fat diet showed remarkable increase in body weight (86.1%), blood glucose level and all lipid parameters with dramatic decrease in HDL. Maximum decrease in weight (28.6%) was noticed in rats received 50% artichoke followed by 25.7% and 16.3% decrease in rats received 50% barley meal and 6% chitosan respectively. Lipid profile and glucose levels have also decreased following treatment. The highest recovery was observed in the group received J. artichoke flour while barley meal had medium recovery followed by chitosan. Histopathological examination confirmed the previous results. In conclusion, food products containing J. artichoke are superior to those containing barley or chitosan for management of overweight and/or obesity.

PC47

Preliminary results of a human osteomyelitis model of Methicillin-resistant Staphylococcus aureus in rabbit

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Abstract
The rabbit model for osteomyelitis has not been adapted yet to study strains from human. Taking into account the fact that Romania has the highest incidence of Methicillin-Resistant Staphylococcus aureus (MRSA) from Europe, we have tried to create a humanize model of osteomyelitis on rabbit inoculated with a MRSA strain isolated from a human patient with clinical signs.

This paper aims to present the evaluation of installation and evolution of the induced osteomyelitis on rabbit by clinical, hematological, radiological, necropsy and histopathological examinations. We tried to comply with the 3Rs principles and based on the historical results and created only two groups of 15 animals which received two types of MRSA in two concentrations: 5x104UFC/ml and 5x106UFC/ml injected (0,1 ml) and additional materials used as foreign bodies (inoculated cotton meshes) in bone defects, created in the left tibia under total anesthesia. As control we used the right tibia. The follow-up period was 37 days, during which the animals were clinically examined daily and when indicators of pain were identified (abscesses and open wounds), analgesics were administered at the dose and by a route appropriate for the study and species. Daily monitoring of alterations to general wellbeing, body temperature, and weight was essential for establishing humane endpoints in this study.

The results shows that MRSA human clinical strain can reproduce the acute and chronic osteomyelitis in rabbits proving that it is a good experimental model in developing new treatments.

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PC48

Preliminary assessment of Cu ion solution – based treatment against chronic osteomyelitis

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Abstract
Introduction: In this work, an animal model was used to study a new therapy based on copper ions against chronic osteomyelitis.
Aim: To assess the potential of copper ions saline solution to treat chronic osteomyelitis in rabbit tibia.
Materials and Methods: Osteomyelitis was induced in rabbit tibia of 40 animals by infection with Staphylococcus aureus ATCC6538 using cotton meshes soaked in bacterial suspension. 45 days after inoculation, the meshes in all rabbit tibias were removed and 28 rabbits were inoculated with 166 ppm Cu-SS. Animal monitoring was made by clinical observation, microbiology, histology and radiology over 120 days. Analgesic administration after surgical intervention and also when limping appeared, as well as humane killing on measurable loss of appetite and/or lethargy were undertaken.
Results: Although none of the tibias were found positive to the inoculated bacteria on the 45th day of investigation, radiological and histological investigations revealed clearly the presence of

infection and of specific osteomyelitis lesions. Radiological assessment of the rabbits showed smaller modifications of bone architecture in the treated group than in the control, 120 days from the start of experiment. Histology revealed intra-osseous inflammation and periosteal deformation as main lesions in the untreated group on Day75, lesions which almost disappeared at the end of experiment (Day145). The treated group showed intra-osseous inflammation in less than 30% rabbits only.
Conclusions: The results represent the synergy of immune system of rabbits and the antimicrobial effect of treatment used. Further work will target assessment of proposed treatment against acute osteomyelitis.

PC49

Preliminary evaluation of Cu ion solutions for the control of severe bone infection

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Abstract
Introduction: Bone infection prevention is generally approached by debridement together with administration of systemic antibiotics.

The purpose of this work was to find additional prevention procedures with the least invasion possible. Our study involved saline solution enriched with copper ions (Cu-SS) as a potential agent for infection control.
Aim: A preliminary study on the effectiveness of copper ion enriched saline solution against infected rabbit tibia was undertaken.
Materials and Methods: Two groups of 10 and 5 rabbits, respectively, were infected with Staphylococcus aureus impregnated cotton meshes, followed by inoculation of 0.2 ml Cu-SS of 40 ppm concentration. Disease progression was monitored by standard analysis over 45 days. Pain alleviation was undertaken by administration of painkillers and humane killing in cases where this was imposed by the general state of animals such as measurable loss of appetite and/or lethargy.
Results: Radiological examinations revealed modifications of bone architecture in both rabbit groups with slightly less severe lesions in the treated group. Histology confirmed these results, with necrosis present in 4 rabbits out of 5 in the control group, and only 2 out of 10 rabbits in the treated group.
Conclusions: Overall, it could be concluded that Cu-SS is potentially effective as a preventive measure in the management of infection. Although the results were not spectacular, it should be pointed out that the treatment was administered under severe infection maintained by a foreign body soaked in staphylococcus suspension. Further work will target improvement of the animal model for preventive treatment.

PC50

Differences in fatness and microbiome composition between mice selected oppositely for open field activity

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Abstract
Properly conducted selection leads to changes in the improved trait. In many cases, parallel changes are also observed in non-selectable traits. Two lines of mice – passive (B) and active (A) selected oppositely to the activity in the open field (OF) test were derived from the same parental population. The only selection criterion considered in the breeding of these animals is OF behaviour. The animals are kept in a conventional animal facility, the same breeding room, fed the same complete feed and subjected to the same breeding activities. During the breeding, we observed that the animals from the passive line have a higher body weight than the mice from the active line. Analysis of feed intake showed that animals from the passive line consume less feed, and at the same time are much more obese compared to mice in line A. They have also increased blood cholesterol level but similar to those of active mice glucose level. Analysis of the intestinal flora of animals from both lines showed that the mice of the passive line are primarily infested with microaerophilic fecal Streptococci, Lactobacillus, Helicobacter and fecal anaerobes. In mice from the active line, mainly mesophilic organisms were found, including Gram – positive cocci and Enterobacteriaceae. The change in the composition of the bacterial microflora may be caused by genetic background changes that occurred during the selection of the studied lines. It can also not be ruled out that microbial differences affect animal behaviour.

PC51

New inbred strains of mice derived from lines selected oppositely for body weight

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Abstract
The animal facility of the Department of Genetics and Animal Breeding, Warsaw University of Life Sciences, Poland, maintains lines of mice selected oppositely for body weight at weaning: L (light) and C (heavy). These lines come from the same base population, which was formed by crossing four inbred strains: A, C56BL/6, BALB/c and BN/a. A group of these mice was moved to the Animal Breeding Facility at the Cancer Centre and Oncology Institute, Warsaw, Poland. Developing of inbred strains from both lines started in 2008. Animals from the 126th generation were used as the first sets of parents. At the beginning, we had 55 parental pairs of full siblings from the L line and 50 pairs from the C line. Currently, after 20-23 generations of inbreeding (depending on strain) we receive six and seven strains derived from the C and L lines, respectively. These strains remained similar to the parental line in terms of selected trait—mice derived from the L line are almost twice as small as mice derived from the C line. Conversely, we noticed a considerable variation in other, non-selected traits: the new strains differ significantly in reproduction, fatness and behavior. We also observed differences in the incidence of spontaneous tumors. The very first tests of genetic polymorphism confirmed that each strain possesses the unique genetic profile. We consider that new parallel strains of mice can be a new useful tool in analysis of genetic background of quantitative traits.

PC52

Lines of mice selected oppositely for open field activity

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Abstract
Lines of mice selected for divergent behavior based on an open field (OF) test are maintained at the Animal Breeding Facility at the Cancer Centre and Oncology Institute, Warsaw, Poland. Lines A (active) and B (passive) were bred from this same basal population. The following method is used for behavioral evaluation and selection: at 90 days of age an open field test is carried out, during which mice are observed for 3 minutes in an arena (60×60 cm, divided into 36 fields). The following events are recorded: numbers of fields crossed, rears, fecal boli, urination acts and grooming activity. The results are summarized as a cumulative score for each mouse. The mice with the highest (A) and the lowest (B) cumulative score are selected for further breeding. The overall mean score for parental population was 85.75. After 26 generations of selective breeding, the mean scores were 357,65 and 1,00 in the A line and B line, respectively. ''Active'' mice exhibit great activity in the all regions of the open field arena, a high number of rears, and a low number of fecal boli. ''Passive'' mice show much reduced movement, but an increased amount of defecation was noted. In both lines there were no significant differences in the OF test results between males and females. We suggest that mice from the active line (A) can be a model of psychomotor hyperactivity, while the passive line (B) may be a potential model of severe anxiety and/or depressive disorders.

PC53

Preclinical mouse tumor models: Challegenes and perspectives at the Center of Molecular Immunology

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Abstract
During the last decades the mouse models for cancer serve as a valuable tool not only for understanding the basic tumor biology but also for the development and validation of new tumor intervention strategies as well as for the identification of markers for early diagnosis. A model is a simple representation of a complex cancer system, however, the good selection of a validated and predictive animal model is essential to address the clinical question. Several therapeutic approaches have development by the Center of Molecular Immunology (CIM). The treatments have demonstrated their antitumor effect in different primary and/or metastases models: mammary carcinoma (4T1) in BALB/c; melanoma (MB16F0) and Lewis lung carcinoma (3LL-D122), both in C57BL/6 mice. The endpoint experimentation provided by our CIM-IACC (Institutional Committee for the Care and Use of Laboratory Animals). Here, the current challenges and limitations of animal models are discussed with a focus on metastatic disease. First, the data confirm that the results are associated with the primary tumor or with metastasis, depending on the tumor model which it does not come close to the complexity of cancer. However, the combination therapies increase the antitumoral effects on metastatic models, in terms of survival. In conclusion, to get closer the clinical scenery, we focus on the modelling of clinically relevant metastatic models (orthotopic tumor transplantation and surgical removal of the primary tumor) to investigate the mechanisms of tumor progression and metastasis.

PC54

Breast cancer experimental model induced by 7,12 Dimethylbenzanthracene

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Abstract
Breast cancer is a global public health issue as it is the most frequently diagnosed malignancy in women in the Western world and commonest cause of cancer death. 7,12 Dimethylbenzanthracene (DMBA) is considered to be one of the etiologic factors of malignant neoplasms in humans. It is present in cigarette smoke, coal, burned wood, coal tar and gasoline and diesel engines. It has been assigned various toxicological, immunotoxic, mutagenic, teratogenic and carcinogenic effects. DMBA has also been used to chemically induce mammary carcinogenesis in experimental models with Sprague-Dawley ratsinducing hormone dependent tumors that express estrogen and progesterone receptors.

Sprague-Dawley female rats were orally administered with 65 mg/kg of DMBA diluted in olive oil, at 55-57 days of age. At 15 weeks after carcinogenic induction, tumors started to be detectable by mammary palpation. At 27 weeks, all animals were euthanized under anaesthesia. A full necropsy was performed and all tumors were excised, measured, weighed and characterized macroscopically. This work was approved by DGAV.

The carcinogenesis initiates at 64-70 days of age, 14 days after administration of DMBA. After initiation, the carcinogenesis can cause benign lesions such as cysts, adenomas, alveolar hyperplasias and fibroadenomas or malignant lesions with different histological patterns, for example, papillary, cribiform and comedo.

We consider this a promising model because the mammary tumors are easily induced, the experimental protocols are defined, the animals developed a high number of tumors, and tumors were similar to those found in humans.

PC55

Intravital imaging and Large-scale single-cell resolution in 3D to visualise cancer progression

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Abstract
Large-scale single-cell resolution in 3D (LSR-3D) and intravital imaging are fast growing technological advancements, becoming widely applied in the biomedical scientific field. In cancer research, we are able to use imaging data to create a more complete overview and single-cell profile of tumour behaviour, compared to conventional methods typically used. Intravital imaging allows us to perform experiments over an extended length of time to follow cancer progression and gather live information. Moreover, intravital imaging can be followed up with LSR-3D imaging to gain in-depth data from extracted tissue and further enhance the volume of information gathered.

Here we present several techniques used in our lab to study cancer biology via advanced imaging. Using different types of imaging windows, such as cranial and mammary windows, we can study tumour progression from initial onset, through cell migration and initiation of metastasis for different types of cancer. We have implicated multi-colour lineage tracing to visualize the interaction of tumour with healthy tissue and follow the progeny of single tumour cells overtime.

Data regarding tumour morphology, invasion into healthy tissue and clonal expansion over time can help identify novel targets for therapeutic intervention. Furthermore, with the use of new intravital imaging techniques, single animals should allow us to gather data that was previously gathered from multiple animals, causing a reduction in animal use.

PC56

Development of in vivo imaging methods for monitoring modeled TNF-mediated diseases

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Abstract
TNF transgenic animal models are valuable tools for our understanding of the pathogenetic mechanisms driving inflammatory bowel disease and inflammatory polyarthritis, and the evaluation of novel therapeutics. The current common practice on following up modeled disease is based on both disease activity indices (gross evaluation, weight monitoring and blood tests) and histopathological evaluation of inflammation in affected tissues following euthanasia, in a time-point dependent manner. In this study, we employed bioluminescent-based imaging methods that detect inflammation in situ in TNF-mediated disease models. We aim to develop an accurate, non-invasive method to monitor the inflammatory status in situ in order to provide a platform of direct and objective evaluation of disease progression leading to animal number reduction.

In specific time points, hTNF-tg (spontaneous model of chronic arthritis) and $TNF^{\Delta ARE}$ (spontaneous model of chronic arthritis and ileitis) mice were subjected in luminol and lucigenin i.p. injections for imaging of acute and chronic stages of inflammation respectively. Following injections, bioluminescence images were acquired and analyzed (In-Vivo Xtreme).

We show that the total bioluminescent signal in the standardized regions of interest fully corresponds with the inflammatory process over time, compared to the clinical indicators and the histopathological examination. Therefore, we suggest that the in-vivo imaging of inflammation-induced bioluminescence can be efficiently used for monitoring disease of the hTNF-tg and $TNF^{\Delta ARE}$ models, leading to an unbiased and precise evaluation of pathogenic activity under the minimum number of mice and avoiding euthanasia during early and moderate stages of the disease.

PC57

A new tool for measuring tumors in mice improves health reporting and refines welfare

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Abstract
Monitoring mice with tumor xenografts is essential to animal welfare in oncology research. Husbandry technicians report masses if large, ulcerated, and/or impeding movement. Our veterinary technicians then observe the animal, measure masses with calipers, and complete an electronic health report which emails research personnel. Cases are rechecked at least once weekly to ensure resolution. Reporting was historically inconsistent and complicated by the subjective interpretation of a ''reportable'' mass. Observing, reporting, documenting, and monitoring animals was very time consuming. Mice were often reported unnecessarily early, and then required weekly monitoring. In other cases, tumors at criterion size for intervention were not being reported. A small stainless tool with 1 and 2 cm diameter holes was developed to provide more objective tumor size information. By holding the tool against the skin and seeing how much space the mass occupied, technicians easily and accurately assess whether a mass is at or near to IACUC accepted limits (2 tumors at 1cm or 1 at 2cm). Data was collected 40 days before and 44 days after implementation. Newly reported cases decreased by 50%, from 50 to 25 cases, and correspondingly the number of weekly rechecks decreased significantly. Compliance by researchers also improved with more timely euthanasia of mice, a result we attribute to them receiving fewer but more targeted emails that now reflect mice needing immediate attention. The husbandry technicians feel more confident that they are reporting appropriately, and significant time savings is shared by all.

PC58

Replacement of the teratoma assay for testing pluripotency and malignancy of human-pluripotent stem cells

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Abstract
The teratoma assay in mice is currently the gold standard to determine pluripotency and malignancy of human pluripotent stem cells (hPSCs). This assay is heavily criticised since it is animal-dependent, laborious and only qualitative.

In order to find an *in vitro* alternative to evaluate pluripotency of hPSCs, a side by side comparison of several *in vitro* tests and the teratoma assay was performed. The results showed that a combination of a bioinformatic assay based on the transcription profile of undifferentiated stem cell lines (PluriTest) and a quantitative PCR test based on analysis of stem cells after a short differentiation protocol (ScoreCard) were able to predict pluripotency. Remarkably only the teratoma assay gave information on malignancy potential.

Tumors derived from stem cells are similar to human germ cell tumors (hGCTs). hGCTs in human patients are diagnosed by blood analysis of specific circulating microRNAs. Therefore we investigated if these microRNA families would be found in mice injected with human stem cells. Mouse plasma microRNA profiles were monitored over time during xenograft formation. miR-371 family members were able to faithfully report undifferentiated/malignant elements in the xenografts at early stages of tumor development. This miRNA based test allows a tumorigenicity *in vivo* test with smaller tumors and shorter duration of the assay.

In conclusion the teratoma assay can be replaced for determining pluripotency; regarding malignancy we have developed a microRNA based test able to make an early and quantitative diagnosis of malignancy; this represents a significant refinement of the traditional teratoma assay.

PC59

Patient-derived and cell line xenograft growth in the B6;129-Rag2tm1FwaIL2rgtm1Rsky/DwlHsd (R2G2) mouse model

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Abstract
Herein we describe growth of multiple patient-derived (PDX) and tumor cell line xeno- (CDX) and allo-grafts in the R2G2 immunodeficient mouse model. Colorectal PDX tissue was subcutaneously implanted into R2G2 and NSG mice. Growth was comparable between the R2G2 and the NSG mouse models. Head and neck PDX 626 and 635 was transplanted in 2.2 mm^2 tissues into 4 sections of each of 2 R2G2 mice each ($n = 2$/PDX), and 100% of mice developed either one or two tumors. The human esophageal adenocarcinoma OE33 cells were implanted into the left and right flanks of three each of R2G2, Athymic Nude and SCID mice. There was a 100% take rate in R2G2 mice, 0% in SCID mice and 17% in Athymic Nude mice. The human esophageal adenocarcinoma FLO1 cells were injected into both flanks of R2G2 and SCID mice, with a take rate of 100% in R2G2 mice. Human gastric adenocarcinoma AGS cells were implanted in both flanks of four each of R2G2 and SCID mice. The take rate was 75% in R2G2 mice and 0% in SCID mice. Head and neck squamous cell carcinoma SQ20b cells were implanted in twenty R2G2 mice and take rate was 90%. The mouse colon carcinoma CT26 cells and mouse B cell

lymphoma both had a take rate of 100%. All studies were IACUC approved. Animals were monitored daily for symptoms of pain and distress. Any animals with a tumor larger than 2cm, with an open sore or inhibited movement were removed from the study and euthanized. These data provide evidence that the R2G2 mouse model is a valuable tool for oncology programs including cell line tumor models research, with high take rates and quick growth of allogeneic models.

PC60

Chemotherapeutic tolerability and Estrogen dose response in the B6;129-Rag2tm1FwaIL2rgtm1Rsky/DwlHsd (R2G2) mouse model

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Abstract
The literature supports better tolerability of DNA damaging oncology treatments for models that do not carry the *SCID* mutation. We have already reported in a white paper that the R2G2 mouse model is more tolerant of whole body radiation than a model with the *SCID* mutation. Herein we describe a study examining chemotherapeutic tolerability of common DNA damaging oncology drugs including 5-fluorouracil (5-FU), doxorubicin (Doxo), and cyclophosphamide (CTX) (n = 10 per group). 5-FU was given at 30, 60 or 100 mg/kg, intraperitoneally, twice weekly for five weeks. Doxo was given at 2 or 5 mg/kg, intraperitoneally, once weekly for three weeks. CTX was given at 100 or 140 mg/kg intraperitoneally, once weekly for three weeks. Results show that the R2G2 mouse model tolerates higher doses of these chemotherapeutic drugs than doses found in the literature for *SCID* models. Exogenous estrogen tolerance is another common concern in oncology research as some immunodeficient mouse models cannot tolerate the subcutaneous estrogen pellets, developing negative secondary effects resulting in removal from study. We performed an estrogen pellet dose response study using four doses of 60-day release 17-β estradiol pellets at 0.18, 0.36, 0.72, and 1.7 mg/pellet (n = 10 per group). R2G2 mice show dose dependent effects of estrogen on morbidity. In conclusion, these data support that the R2G2 mouse model may be a good alternative to *SCID* models when administering DNA damaging chemotherapies or when estrogen supplementation is required for xenograft growth.

PC61

Protective effects of colocasia esculenta leaves extract on in vivo experimental models of hepatotoxicity

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Abstract
Colocasia esculenta (L. Schott) has common names as Cocoyam, Taro, Elephant ear and Dasheen. In Nigeria, the tubers are used as carbohydrate staple food whereas the leaves are used traditionally, especially in rural areas, to cure various diseases including liver ailments. Due to the importance of validating the efficacy of herbal medicines claims, the present study was designed to evaluate the hepatoprotective effects of C. esculenta leaves which were collected from different farm sites in Enugu metropolis, Nigeria. Crude aqueous extract was prepared from dried powdered leaves of the plant material. Three in vivo models of hepatotoxicity in rats to establish chemical, drug and high-fat diet-induced liver injuries using thioacetamide (TAA), paracetamol (PARA) and lard-enriched diet (LED) respectively in adult rats were performed. Biochemical estimation of serum levels of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphate (ALP) as well as microscopical examination of excised liver tissues were performed at the end of the experiments. Results revealed that TAA, PARA, and LED significantly increased the levels of ALT, AST and ALP ($p<0.05$). Histomorphological changes observed in the excised liver tissues were consistent with toxicities caused by the hepatotoxicants. However, treatments with C. esculenta leaves extract significantly prevented the TAA, PARA, and LED-induced elevated levels of the serum biochemical parameters ($p<0.05$). These results were supported by the histopathological observations which showed good preservation of the liver histoarchitecture of C. esculenta-treated rats. Overall findings in this study suggest that Colocasia esculenta leaves possess hepatoprotective activity against in vivo models of hepatotoxicity.

PC62

Effect of colocasia esculenta extract on adipocytes cytomorphometry of high-fat diet-fed rats

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Abstract
The objective of the present study was to evaluate the effect of crude aqueous extract of Colocasia esculenta leaves on the cytomorphometry of adipocytes from visceral adipose tissues of high-fat diet-fed rats. Preliminary phytochemical analysis and acute toxicity testing of the extract were conducted. Eighteen (18) healthy adult male rats (Wistar strain) were divided into three

groups of 6 rats each as follows: normal, high-fat diet [HFD] control, and HFD + 400 mg/kg C. esculenta extract (CEE) groups. Rats were fed a HFD [20% Lard, w/w] for 10 weeks. Body weights and adipose tissue weights of all animals were measured after a 28-day CEE treatment [Day 43 – 70] via oral gavage. Blood samples were obtained via retro-obital puncture for serum total cholesterol (TC) and triglycerides (TG) analyses. Paraffin-wax embedding technique for light microscopy was employed for histological processing of excised adipose tissues. Adipocytes cytomorphometry was conducted after identification of tissue sections using Haematoxylin and Eosin staining technique. Phytochemistry revealed abundant amounts of saponins and alkaloids. Oral LD50 of >5g/kg body weight was obtained from the acute toxicity testing. Results showed significant reduction ($p < 0.05$) in body weight gain, total adipose tissue weights, adipocyte diameter and TG levels in CEE-treated rats compared to HFD-control rats. Histological examination of the adipose tissues from HFD-control rats revealed hypertrophy of the adipocytes whereas normal adipocytes sizes were observed in CEE-treated rats similar to normal control. In conclusion, our data suggest that CEE inhibits HFD-induced fat accumulation and weight gain in albino rats.

PC64

Loss of Ing3 expression results in growth retardation and embryonic death

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Abstract

The candidate tumour suppressor inhibitor of growth 3 (*Ing3*) belongs to a family of histone modifying proteins involved in regulating cell growth, senescence, apoptosis, chromatin remodelling, and DNA repair. It is a stoichiometric member of the minimal NuA4 histone acetyl transferase (HAT) complex consisting of Eaf6, Epc1, Ing3, and Tip60. NuA4 HAT is responsible for the transcription of an essential cascade of proteins that are involved in development and in tumour suppression. Ing3 has been described as being associated with head, neck, and hepatocellular cancers. Nevertheless, its putative tumour suppressor status has yet to be fully established as recent studies suggest a pro-metastasis role in prostate cancer progression. We here describe an insertional mutant mouse strain where an UbC-mCherry expression cassette has randomly integrated into the *Ing3* locus, resulting in depletion of Ing3 protein expression. Homozygous mutants survive up to E10.5, display embryonic growth retardation, and are approximately half the size at E10.5 compared to hemizygous and wild type mice that develop normally. μCT analysis revealed a developmental defect in the closure of the prosencephalon in homozygous E10.5 embryos. This is consistent with high Ing3 expression in the epithelial line of the brain in both, wild type and hemizygous mutants.

Our data provide strong evidence that Ing3 is an essential factor for normal embryonic development, fulfilling a fundamental role in fetal brain development.

PC65

Refining the Catwalk™ XT gait analysis by clicker training

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Abstract

The Catwalk™ XT gait analysis is a behavioral test used to assess various motoric parameters like coordination, stride lengths, bodyweight distribution and velocity in rodents. Animals are required to pass voluntarily through a straight glass bottom gateway to make the footprints visible to the camera system.

Many animals fail to do so as they do not successfully complete the required distance in a constant speed, leading to an increased number of animals needed in the study. Using clicker training we apply two of the principles of 3R, reducing and refining, by adapting animals to the environment of the test apparatus and training them to pass through the gateway as fast as they can in order to produce quick and stable results. Over a two week training period animals learn to accomplish the task and are then compared to a native control group. Aim of the study is to decrease animal numbers needed for the test and reducing stress for the ones used by habituating them to the test conditions. In specific parameters, this leads to fewer uncompliant runs and a lower run duration compared to untrained animals, as our experiments showed.

For the scientist it can mean saving time and financial resources by preparing fewer animals for the test.

PC66

A new non-invasive cardiorespiratory telemetry jacket for rodents!

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Abstract

Radio telemetric devices enable monitoring activity and physiological parameters in conscious and unrestrained animals. Telemetry contributes to reducing the number of animals used, refines procedures and provide data considered to be more predictive. Whereas several jacketed telemetry systems are available and now commonly used in large mammals (dogs, monkey, pigs) cardiorespiratory monitoring in small mammals still requires surgery or restraining.

We developed a cardiorespiratory monitoring jacket that embeds external ECG recording, activity monitoring and respiratory inductive plethysmography strips (RIP). In order to validate this new technic, we conducted a study to compare physiological measurements from the jacket with reference methods: Unrestrained Whole-Body Plethysmograph chamber (UWBP) and an implanted ECG telemetry device. This study was performed on 6 telemetered Sprague-Dawley (505 ± 28 g) males equipped with a jacket and placed in a plethysmograph.

All animals kept the jacket for 2 hours without any sign of struggling. Cardiac (ECG, heart rate) and Respiratory Rate (RR) parameters were simultaneously recorded for 1 hour in rest conditions. Data were averaged every 15 seconds. We found good agreement between jacket versus reference measurements using the Bland Altman method to calculate 95% confidence intervals: for HR ±24 bpm (n = 1129) and RR ± 18 bpm (n = 331).

In conclusion, in small animals, a cardiorespiratory monitoring without surgery and restraining is possible by using a non-invasive jacket. This new cardiorespiratory monitoring technic could become an alternative to refine and simplify procedures in several fields such as endpoint monitoring, physiopathology, safety pharmacology and toxicology studies.

PC67

A reliable method for collecting saliva samples in mice

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Abstract
Collecting saliva samples in an efficient and reliable way in small rodents such as mice is challenging. Saliva can be a valuable sample for determining enzyme activity, stress assessment and even a source of DNA. The goal of this study was to develop a method for collecting consistent amounts of saliva from mice. Several approaches for saliva collection were tested and the method yielding the highest saliva volumes was validated by assessing alpha-amylase activity as a stress indicator, compared to using blood corticosterone levels. The results showed that the developed method generated consistent amounts of saliva exhibiting intact enzymatic activity.

PC68

Tip matters: Jugular vein catheter patency in Sprague-Dawley rats

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Abstract
Surgical implantation of jugular vein catheters (JVCs) facilitates central venous access in preclinical models. Use of JVCs can enhance animal welfare and potentially reduce the number of animals needed, along with refining study design to get better, translatable data. Patency, or bi-directional flow, defines the functional life of a JVC; therefore, it is important to examine potentially contributing factors, such as catheter tip shape and maintenance. Rat JVCs are widely accepted and used, however, design and manufacturing process vary among institutions with limited scientific evidence surrounding the efficacy of either factor. This study compares patency of JVCs from three different manufacturers. Each JVC is made of 3-french polyurethane; two have a rounded tip, and one has a bullet tip. Fifteen male 300g Sprague Dawley rats were randomly assigned to one of three groups (N = 5) and catheterized via the right jugular vein. Catheter patency was evaluated every 3-4 days for 28 days. Catheters were deemed fully patent if blood was withdrawn during the initial attempt. If blood was not immediately withdrawn, 0.1 ml saline infusion was followed by a second attempt. If successful, catheters were considered partially patent. If unsuccessful, catheters were considered non-patent. After 28 days, 100% of one rounded tip, 60% of the other rounded tip, and 80% of the bullet tip catheters were considered patent. Bullet-tip catheters showed repeated instances of partial patency and had the first instance of non-patency. In conclusion, this pilot study indicates rounded-tip design is superior to bullet-tip design in maintaining catheter patency.

PC69

Mouse temporal haematology blood profile: Combined refinements substantially reduces animal numbers

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Abstract
Haematology analyses is a critical component of pre-clinical toxicology studies as well as providing important pharmacodynamic biomarkers in early drug discovery research. Most automated haematology analysers require a blood sample volume in excess of 160μl of whole blood. The requirement for this amount of blood limits the opportunity for repeat blood sampling in mice (due to their small size and blood volume) and for acquiring a combined haematology and clinical chemistry profile from pre-clinical in vivo studies. Murine sampling in particular is prone to loss of data as a result of clotting or blood volume issues. Comparative and Translational Science, GlaxoSmithKline UK refined a combination of micro sampling techniques and automated capillary haematology analyses to successfully achieve a temporal murine haematology profile. Using a double cohort approach, we were able to generate 12 scheduled bleeds (11 tail vein and 1 terminal) across a three week study period with a 96% success rate for reportable haematology results. The combined refinements reduced the animal number required by over 80% while increasing the robustness of the study by enabling integrated results from the same mouse. All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act

1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.

PC70

Capillary micro sampling (CMS) techniques in rodents, a reduction and refinement method

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Abstract
The CMS technique has many advantages over other blood sampling methods in rodents. It does

not require sedation or anesthesia of the animals prior to sampling. In addition, the sample volume is small (10 ul) and hence many samples can be taken per animal, which reduces the number of animals per experiment. In conclusion, the CMS technique in rodents is a reduction and refinement method that delivers high quality data.

PC71

Suitability of human point-of-care analyzer for measurement of blood lipids in mice

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Abstract
Limited blood volumes of small rodents pose challenges for repeated blood lipid measurements. Using point-of-care analyzers for laboratory animals can be considered as a refinement since less blood is needed for repeated measurements. The aim of this study was to evaluate the applicability of a point-of-care human cholesterol testing analyzer for measurements of blood lipids in mice.

We used spontaneously hypercholesterolemic ApoE KO and C57Bl/6J male mice. ApoE KO mice were fed soya free chow (SF), western (WD) or high-fat diet (HFD). The C57Bl/6J mice were kept on SF diet. Total cholesterol (TChol), HDL and triglycerides were measured by CardioChek plus 2700 analyzer (PTS diagnostics) from whole blood samples. For comparison, we also analyzed TChol and triglycerides with enzymatic colorimetric assays (ECA) using frozen serum samples.

TChol values measured with CardioChek were above the upper detection limit (>10,36 mmol/l) in all ApoE KO mice in HFD group. Only one ApoE KO mouse in WD group and four ApoE KO mice in SF group had TChol levels within the detection range. TChol values were below the lower detection limit (<2,59 mmol/l) in all C57Bl/6J mice. TChol and triglycerides from ApoE KO and C57Bl/6J mice from different diet treatments were measurable with ECA. Triglyceride values between the two analysis methods were comparable.

CardioChek point-of-care device can be used for analyzing HDL and triglycerides in mice. However, it is not suitable for measuring TChol unless the detection range is adjusted to detect higher and lower TChol levels.

PC72

Play-time as a refinement in long-term pharmacokinetic studies in rats

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Abstract
Pharmacokinetic (PK) studies are performed to determine the pharmacokinetic properties of compounds and progress the best ones to the development of new treatments. Rat PK studies may be performed in surgically cannulated animals for serial blood sampling when there are numerous time points in a short period of time or when sample volumes are large. However, rats need to be housed singly to avoid damage to catheters, which can be stressful as they are highly social animals[1,2]. Therefore, to improve animal welfare during long-term surgical PK studies, male Sprague-Dawley rats were allowed to interact regularly under supervision for half hour, 2-4 days/week for 11 weeks. A large cage was furnished with elements for rats to climb, hide and forage, while allowing permanent supervision to ensure catheters would not be damaged. Additionally, the person supervising carried out playful handling with the rats that approached their hand[3]. Rats interacted with each other and used all items of the cage throughout the half-hour and all weeks of the study. Play-time was considered valued by the rats: we observed an increase in activity when taken out of the cage, voluntary movement from the holding cage to the play-cage and 50kHz vocalizations indicative of positive affect[5]. Rats also showed a positive attitude towards staff: they tended to cluster more near the supervisor and showed voluntary approach to their hand[4]. No aggression or any other negative behaviour was observed during the study. In conclusion, play-time was considered a positive refinement for long-term rat PK studies.

PC73

Evaluation of infrared thermography for temperature measurement in adult male NMRI nude mice

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Abstract

"Temperature monitoring during critical care provides important data required to guide treatment delivery. Body temperature is an easily quantified clinical parameter that can yield much information concerning the health of an animal. In research settings, temperature has been adopted as a means to judge humane endpoints. Therefore, reliable, noninvasive, and inexpensive methods for temperature monitoring are becoming a necessity in research laboratories. This study aimed to determine the accuracy and agreement of using an infrared camera as an alternative method of temperature measurement in mice and to compare the accuracy of this noninvasive method with established subcutaneous, intraperitoneal, and rectal techniques.

Measurement of body-surface temperature by using an infrared camera was compared with these 3 established methods in male NMRI nude mice (n = 10; age, 10 mo); data were obtained 3 times daily over 14 d. Subcutaneous temperatures were measured remotely by using a previously implanted subcutaneous temperature transponder, after which temperature was measured by using noncontact infrared thermometry and a rectal probe. Measurements from intraperitoneal data loggers were obtained retrospectively. The data show that using an infrared camera provides a simple, reliable method for measuring body temperature in male NMRI nu/nu mice that minimizes handling and is minimally invasive."

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PC74

Telemetric measures in a rat model with intracranial tumor

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Abstract

Background: In rodent cancer models a fixed weight-loss of 20% threshold and deterioration of clinical scoring is often not sufficient as humane endpoint. We recently proposed a body weight analysis function, which can be used as a reliable tool for endpoint determination in intracranial tumor models. In the current study, we tested whether contactless and continuous measurements of heart rate, temperature and activity via subcutaneous implanted transmitter devices would be useful to further refine humane endpoint criteria in an intracranial rat glioblastoma model.

Method: By minimal invasive surgery under general anesthesia a telemetric device was subcutaneously implanted in male BDIX rats (n = 8). After 4 weeks of recovery, glioblastoma BT4Ca cells were stereotactically implanted into the prefrontal cortex. Starting with the day of surgery heart rate, body temperature and activity were measured until the end of the experiment.

Results: Implantation of BT4Ca cells reliably induced fast growing tumors with a survival time of 12-16 days. On average, body temperature, heart rate and heart rate variability were reduced on the last two days before perfusion without deterioration of clinical score. Furthermore, activity gradually decreased over the last 8 days. On individual animal level, however, high variability was seen and no clear criterion could be defined for endpoint determination.

Conclusion: On the level of the individual animals, telemetric measurements were not superior for endpoint determination than our weight analysis function. Additionally, our endpoints were confirmed, as all animals showed no unusual measurements until reaching the endpoint.

PC75

Development of a needle length reductor for intracerebral injection: Improvement, standardization and safety

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Abstract

Sanofi Pasteur performs quality control tests on animals. Some of them require intracerebral injections which is challenged about the control and the reproducibility. In order to meet injection's expectations (depth ≈ 5mm, tilt = 90°), a stopper has been developed. This device should be added on the needle to ensure the depth requirement (5mm). A study has been realized to identify the accurate injection depth, using medical imaging. This device has proved to ensure the control of the injection in terms of depth and tilt. This control reduces technicians stress: they can exclusively focus on the injected volume and not anymore on the needle position after insertion (human benefit). The injection control also allows a complete standardization of the injection between technicians. It could therefore allow the reduction of invalidity rate for the concerned tests. The stopper offers real benefits in terms of training. Training could be shorter in time thanks to the easy manipulation of stoppers. As part of the 3Rs principle, this device allow the reduction of animals throughout training for tests including intracerebral injection. All these aspects can be expressed into ethical, time and financial benefits. Moreover, a stopper carrier has been developed in order to eliminate the risk of pricking throughout the positioning of the stopper: no contact anymore with the needle all along the test (HSE benefit). For the future, the stoppers and the stopper carrier could be deployed and easily designed for every kind of needle or injections (ex: intramuscular injection).

PC76

Resting state recordings in multi electrode EEG in freely moving rats

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Abstract
Electroencephalographic (EEG) studies in freely-moving rodents are commonly used as a translational tool to predict drug-effects in humans. The direct translation between rat and human is challenged by obvious anatomical differences as well as differences in the states under which the data is recorded. We have set up a resting state EEG (rsEEG) model with a 6-channel electrode configuration in freely moving rats. Wistar (Han) male rats are used. The electrode configuration consists of 4 variable electrode placements, a reference electrode and a ground. The investigated brain regions in our initial experiments included depth electrodes in infralimbic cortex, nucleus accumbens and thalamus as well as a screw electrode in the auditory cortex. However, we may record from other areas in the brain that are accessible when the rat is placed in a stereotaxic frame. The model was implemented with locomotor activity detection via an accelerometer allowing for analysis strictly during periods of either active locomotion or inactivity. We show effects of ketamine, DOI, d-cycloserine, d-amphetamine, and diazepam. Our initial experiments revealed that the rsEEG in general was more sensitive to pharmacological challenges when the rat was inactive but also that ketamine-induced delta activity was found specifically related to locomotion. We conclude that analysing the rsEEG data separately in ''active'' and ''inactive'' states improves quality of data and reveals previously unknown phenomena.

PC77

Pain and stress free urine collection in macaques

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Abstract
Urine analysis plays an important role in assessing animal health and welfare, as well as answering physiological and metabolic questions. A small quantity of urine is often sufficient to perform qualitative analyses. In some cases, a timed sampling is necessary for quantitative analyses in order to obtain data on renal function or metabolite excretion. The method by which urine is collected greatly influences data and their interpretation. Specific precautions must be implemented to reduce pain and distress to the animal, insure overall sample quality, and prevent contamination. Publications report a variety of techniques to collect urine in macaques. However, most of them are highly invasive and based on cystocentesis or catheterization. We therefore investigated a pain and stress free method of urine collection on rhesus and cynomolgus monkeys. For that, we used a system based on a special water-repellent bedding material. This atoxic material is placed on the bottom of the cage with or without the use of nylon fishnet, so that no animal restrain is necessary. Our goal was to test the system and verify that we could easily and reliably check physiochemical and sedimentation urine characteristics during urogenital tract evaluation as well as general health monitoring. Our technique was well accepted from the animals and easy to perform by operators. The quality of the samples was preserved. We are consequently fully convinced that this new method of urine collection could serve when possible as a replacement of invasive techniques.

PC78

The development of thyroid reference values for the common marmoset

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Abstract
Health monitoring is an important concept for all non-human primate colonies. Recognition in changes of the metabolic state enables quick assessments. The aim of our recent research was to analyse thyroid hormone values.

For this, blood from 28 marmosets (17 males, 11 females) averaging an age of 8.3 years (male average 7.8 ± 3.08 years, female average 8.5 ± 4.9 years), spanning from 1 to 16 years old, was used. The body weight on average for female marmosets was 404.4 ± 67.6 g and 395.5 ± 45.5 g for males. The kits by ''BioTrend GmbH'' were used for T3, T4 and TSH evaluation and carried out as described.

As human kits were used, the ELISA was validated for marmosets by testing parallelism as well as recovery and using human serum samples as controls. First results showed that parallelism of male and female marmoset blood samples as well as recovery was present in the assays. The concentration for T3 was at 1.66 ± 0.29 ng/ml in females and 1.55 ± 0.18 ng/ml in males and for T4 61.83 ± 15.52 nmol/l in females and 61.71 ± 11.79 nmol/l in males. There were no significant differences in T3 and T4 values when compared to gender, age, or weight. TSH values were not measurable. However, marmosets with blood lipid variations and growth retardation showed a change in T3 and T4 levels. In summary, T3 and T4 values in common marmosets are measurable and could enable further research towards some metabolic diseases.

PC79

Training with common marmosets – Recognising and understanding particularities

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Abstract
Common marmosets (*Callithrix jacchus*) are a widespread animal model for human biomedical research. To get reliable data, primates need to show cooperativeness in order to reduce stress for both animal and researcher. Stress during routine procedures can be minimised through training, supporting the 3 Rs for animal health. The goal of this study was to assess training principles and their success.

The techniques used were based on clicker training. This method, which is common practice in dog training, needs to be adapted when working with common marmosets, as their behavioural action is fast and they need a larger individual distance to trainer and object, rendering methods such as targeted object introduction useless.

The method of ''free shaping'' has so far shown to be successful. We used target training with tactile differentiable clips to enable health checks and stress free separation. Furthermore, we used stationary targets such as weighing scales or a sieve for weight checks and stress free urine sampling. Resources used for training have to be suitable for the species, for example, the targets should be selected by size and tactile structure, as colour recognition has not yet been fully researched for marmosets. Following their activity patterns, the time of day also has to be taken into consideration for successful training.

In summary, these training methods for primates support cooperative action as well as stress reduction for animal-human-interaction in experimental settings, thus in turn having a positive impact on animal well fair.

PC80

Improvement of animal welfare in a model of combined pharmacokinetics and telemetry in minipig

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Abstract
Acknowledging the 3Rs rule during non-clinical drug development implies using appropriate study designs to address a well-defined scientific question, while taking into consideration animal welfare. The minipig is a large species used in pharmacokinetics and cardiovascular models, as it has similar physiology and cellular receptors to humans. However, this species is highly sensitive to stress and this must be considered when defining and refining study design to limit the possible impact of stress on study data.

We have combined two approaches: surgical implantation of telemetry devices and vascular access port (VAP) apparatuses and an adapted training program with specific restraint equipment. First, refinement of surgical procedures allows concomitant implantation of the telemetry transmitter and the VAP with a single post-operative period. Moreover, the VAP provides easy access for blood sampling with the animal in a natural posture. Secondly, we have developed restraint equipment similar to livestock equipment, in order to respect the pig's prey species behavior, and we have associated a training program with following the target and habituation to the restraint equipment. Using these two approaches, the animals voluntarily come to be sampled, without any handling or stress.

By combining two approaches, we show how appropriate study design taking into account the animal's well-being can improve animal welfare in line with the 3Rs rule, which may positively impact the quality of the data in a model of combined pharmacokinetics and telemetry in the minipig.

PC81

A new method for percutaneous carotid access in pigs using anatomical landmarks

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Abstract
Carotid vascular access is commonly used to perform interventional procedures in the coronary arteries, as well as peripheral vasculature. Both, surgical and ultrasound guided access techniques have been reported. Surgical cutdown can lead to complications like wound infection or dehiscence, while ultrasound systems might not be readily available on site and require experience in imaging to succeed. We developed a simple procedure for percutaneous common carotid access in pigs using a standard Seldinger's technique based on anatomical landmarks. Sixty yorkshire crossbreed pigs with an average weight of 45.7 kg were anesthetized, intubated, and placed in dorsal recumbent position with the fore limbs stretched and secured caudally. The access site was delineated on either side to the sternum's manubrium in the thoracic inlet and prepared aseptically. An arterial needle was advanced and arterial sheaths of sizes 6 to 9 Fr were placed using a standard Seldinger's technique. Afterwards, vascular sheaths were removed and hemostasis was achieved by applying manual pressure at the puncture site. Carotid access was successful in all animals. There was no mortality. We observed no hematoma at the puncture site. The average carotid access time accounted to 1.6 minutes. In 3 animals, the needle punctured the balloon from the endotracheal tube, and re-intubation was necessary. In all animals, the ipsilateral artery was successfully used for a second vascular access after 3, 7, 30, 90 or 180 days. Percutaneous vascular access with the described method prevents the above-mentioned complications, significantly reducing access time, without requiring specialized equipment or skills.

PC82

Improved tunneling technique for long-term jugular catheter placement with a thoracic catheter in pigs

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Abstract
To minimize stress when collecting multiple blood samples, a permanent vascular access is particularly useful in pigs. Eight pigs (2-3 months old, medium weight of 27 kg) were enrolled in this study. Anaesthesia and analgesia were performed according to the standards of the University hospital. After positioning in dorsal recumbency and surgical access the jugular vein was catheterized with medical tubing. Pigs were turned into right lateral recumbency and a thoracic catheter with inner stylet was inserted into an incision on the dorsal neck and fed forward subcutaneously until the tip surfaced in the ventral neck incision. The stylet of the drain was removed and the free end of the catheter was threaded through a hole in the tip of the drain. Then, the drain (with the catheter inside) was slowly pulled out of the dorsal neck incision. Wounds were closed and the catheter secured. Overall, total anaesthesia time was less than one hour, tunneling time was about 15 seconds. Peri-/postoperative analgesia was provided with ketamine, fentanyl and meloxicam. Anaesthesia, catheter placement and recovery were uneventful in all animals. Pathology several weeks later revealed no signs of infection or swelling at the surgical site. We describe an improvement of the tunneling technique for long-term jugular catheter placement in pigs with the help of a commercially available thoracic drain. This simple technique ensures a shorter anaesthesia time and causes less tissue trauma than conventional tunneling techniques which results in faster wound healing and less pain postoperatively.

PD1

Influence of ground transportation on adrenocortical activity in prepuberal female mice

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Abstract
Experimental protocols may necessitate transportation of mice, a potentially stressful event that could confound research results. Adrenocortical activity was determined non-invasively by measuring fecal corticosterone metabolite (FCM) levels, as a stress marker, in prepuberal (3-week old) female C57BL/6J, C57BL/6NCrl, FVB/NCrl, Crl:CD1(ICR), and BALB/cAnCrl mice. On each of 6 days of transport via truck from Charles River Laboratories (CRL), Sulzfeld to the quarantine of the Center for Molecular Medicine, University of Cologne, Cologne (CMMC), 5 female cage mates per strain were weaned together and then kept in stable groups throughout the study. Directly after weaning, fecal pellets from each group were collected on Day 0 (Charles River), as well as on Day 1 and Day 4 (CMMC). FCMs were measured with a well-established enzyme immunoassay. The average duration of ground transportation over 600 km and from packing to unpacking of mice was 7.24 and 22.62 h, respectively. FCM levels were significantly different between days ($p = 0.0138$) and strains ($p = 0.0259$). They increased from Day 0 to Day 1 and decreased on Day 4 in all strains except in FVB/NCrl where values were lowest on Day 1. The results show that weaning and immediate transport of prepuberal mice from the breeding to the research facility led to temporal and strain-dependent increases of adrenocortical activity in 4 out of the 5 strains investigated, which returned to baseline levels 4 days later.

PD2

An easy to introduce protocol to train laboratory rats to voluntary change cages

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Abstract
Cage cleaning is a routinely performed husbandry procedure, which is known to induce stress in laboratory rats. As stress can have a negative impact on well-being and the comparability and reproducibility of research results, stress of laboratory animals should clearly be avoided. Further, the direct contact between rat and animal caretaker during the cage change bears a hygiene risks and therefore possibly negatively impacts the well-being of the rats and the quality of research. Our protocol aims to improve this routinely performed procedure of cage changing. For this reason, we here show a feasible protocol, which enables rats to learn via clicker training and observation to voluntarily change to a clean cage. This allows avoiding stress caused by the physical disturbance and handling associated with the cage change, and concurrently enables the reduction of direct contact between animal and animal caretaker after the training phase is completed. The implementation of clicker training to rats is fast and easy. Rats are generally interested in the training and learn the desired behavior ''cage change through a pipe'' efficiently. Even without training the rats learned to perform the desired behavior by observation, as 80% of the observational learning group showed the cage-change when tested. The training further helps to establish a relationship of trust between trainer and animal. As hygiene and well-being are both very important in animal experiments, this protocol might additionally help to improve high quality research.

PD3

TB-Plex – A high throughput antibody assay for detection of tuberculosis in non-human primates

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Abstract
Tuberculosis (TB) in nonhuman primates (NHP's) is highly contagious and often produces rapid disease. Identification of animals infected with *Mycobacterium tuberculosis* (*M. tb.*) in a timely manner is therefore critical. NHP's are periodically tested using the tuberculin skin test (TST) and/or the Primagam® blood assay. However, these tests lack desirable sensitivity, specificity, efficiency and/or throughput.

TB-Plex, a blood based multiplex immunoassay with seven (7) antigens/assays for detection of antibodies in M. tb. infected animals was developed for routine NHP colony surveillance. Antibody levels were examined in serum/plasma samples from several cohorts of well characterized specific pathogen-free (SPF) colonies. Field trial on sera from ~800 healthy macaques (rhesus and cynomolgus) were used in the validation study to determine specificity of the TB-Plex assay. The cut-offs were calculated and overall specificity of the test was found to be nearly >99%. The panel was screened for sensitivity using experimentally infected rhesus (M. tb. strains: Erdman, n = 6 and H37RV, n = 4) and cynomolgus macaques (Erdman, n = 9). The panel sensitivity was between 80-100% at various time points (8, 12, 16 and 24 weeks) during seroconversion.

In conclusion, a blood based test using serum/plasma was developed and validated which is highly sensitive and specific for screening of M. tb. in NHP's. Also TB-Plex can be performed in a user friendly and high throughput format.

PD4

MagPlex MFIA: A next generation Multiplexed Fluorometric Immunoassay® for serodiagnosis of rodent infectious diseases

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Abstract
Multiplexed Fluorometric immunoassay (MFIA) based on Luminex polystyrene (PS) beads have been in use for more than 15 years for routine serosurveillance of laboratory rodents. A new MFIA using the next generation magnetic MagPlex microspheres was developed. MagPlex beads have several advantages over PS beads including no pre-filtration of samples, no leaky expensive filter plates and improved washing efficiency.

Antigens for several common infectious agents in lab rodents including mouse parvovirus, mouse hepatitis virus, adenovirus, C. *piliforme*, reovirus, rotavirus and Sendai virus were part of the 33 and 28 member mouse and rat MFIA bead panels. Efficacy of MagPlex MFIA was compared to PS MFIA in a validation study using 16 known positive sera from naturally or experimentally infected mice and rats each for one or more of the above mentioned infectious agents. A similar number, 16 known negative sera for each species were used from specific pathogen free colonies. All samples were tested by two different technicians on three different days for a total of six runs.

A total of more than 6000 assays were performed and analytical performance of the rodent MagPlex MFIA assay including selectivity and limit of detection was found to be comparable to or better than those obtained by PS MFIA. Overall diagnostic sensitivity of rodent MagPlex MFIA was >97% compared to >98% for PS MFIA. Diagnostic specificity of both MagPlex and PS MFIA were nearly 100% suggesting that MagPlex MFIA is an acceptable alternative assay for serodiagnosis of adventitious infectious agents of laboratory rodents.

PD5

Assay development for Rodentibacter diagnostics in environmental samples: A novel virulence factor based approach

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Abstract
Hygienic monitoring of laboratory rodents has focused more and more on the analysis of environmental sample material by quantitative polymerase chain reaction (qPCR) assays. This approach not only requires profound knowledge of specific genetic sequences of the respective agents but also demands permanent adaption of the assays to the latest research situation. *[Pasteurella] pneumotropica* was recently reclassified into the new genus Rodentibacter, with *Rodentibacter (R.) pneumotropicus* and *R. heylii* as the most commonly detected species in laboratory mouse colonies. This study aimed at the development of a specific qPCR assay for the simultaneous detection of both agents.

Therefore we chose a novel assay design, based on genetic sequences of the specific virulence factor 'inclusion-body-protein-A'. Assay validation was performed by analyzing the currently described *Rodentibacter* type species and other Pasteurellaceae. It was further validated by testing the assay within four different barrier units, comparing the qPCR results to the findings obtained by traditional analysis of sentinel mice.

The assay was suitable to specifically detect *R. pneumotropicus* and *R. heylii* and discriminate them from other murine *Rodentibacter* spp. In addition, it revealed higher sensitivity for the detection of both agents in environmental sampling material compared to sentinel mice. Bacterial quantification in different sample types showed highest pathogen prevalence in bedding material, cage feces and exhaust air dust filters of the IVC cage systems.

This study describes a novel qPCR for the simultaneous detection of *R. pneumotropicus* and *R. heylii*, which was proven to be

valuable especially with regard to environmental sampling strategies.

PD6

Comparative Approach to Monitoring Rodent Colonies for Infectious Agents

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Abstract
Monitoring rodent colonies for infectious agents is continuing to evolve along with the increased use of individually ventilated caging (IVC) systems, the availability of highly sensitive PCR testing methods and the desire to reduce the use of sentinels exposed to soiled bedding. In this seminar, data from recent real-life changes to the Research Institute of the McGill University Health Centre (RI-MUHC) health monitoring program will be presented. The diagnostic sensitivity of detecting viral, bacterial and parasitic infections of mouse or rat colonies was compared in parallel in traditional tests of soiled bedding sentinels, direct PCR testing of index animals and PCR testing of IVC filters exposed to composite soiled bedding. Advantages, disadvantes and tips for incorporating the new technologies into institutions' health monitoring programs will be discussed through various contamination events that were discovered on the way. This presentation will be of interest to individuals managing or participating in monitoring the health of mouse and rat colonies.

PD7

Harmonization of health monitoring in a decentralized animal facility according to the 3R principle

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Abstract
Aim: The Decentralized Animal Facility of the University Hospital Cologne consists of 11 experimental animal facilities with different construction conditions, hygienic standards and animal species. According to the 3R principle, the health monitoring procedures were newly structured and optimized to reduce the number of used sentinel animals and to save general resources but still complying on the FELASA recommendations.
Methods: 3 different hygienic levels were defined and all procedures within each facility like the frequency of animal import/export and the permitted access of scientists f.e. were critically evaluated. In terms of reducing the number of used sentinels, living animals were completely replaced by in vivum kits in 5 of 11 animal facilities. To evaluate the reduction of general resources all costs concerning the health monitoring were newly calculated.
Conclusion: Regarding the 3R principle, the health monitoring of the Decentralized Animal Facility of the University Hospital Cologne was critically reevaluated. The general costs for the new health monitoring decreased by almost 12%. The number of dead animals was reduced by almost 20 %. To summarize, the installation of hygienic levels and the use of in vivum kits are contributing factors to reduce the number of animals sacrificed for the health monitoring each year.

PD8

Application of the 3Rs to a rodent health monitoring program

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Abstract
1. **R**eevaluate your rodent health-monitoring program at least annually, adjust for adequate surveillance based on the size and specific needs of your animal care, use program, and minimize the number of animals required.
2. **R**eview and incorporate new technology to improve/increase scope and sensitivity of surveillance, and reduce/replace animal usage.
3. **R**ecalculate the cost/benefit ratio of incorporating new technology and review the periodicity or frequency of testing to further reduce costs and the number of animals required.

Application of these three principals to our health-monitoring program resulted in a dramatic reduction in animal use. Implementation of industry standard ''room'' rather than ''rack side'' sentinels, placing one cage of two animals per 60-75 cages for mice and one cage per 30-45 cages of rats, reduced animal usage by >**60%**. Subsequently, our costs for serology decreased by >**25%**. We also incorporated PCR technology to increase the sensitivity and comprehensiveness of our surveillance program, by submitting pooled samples from both colony and sentinel animals. Consequently, because of refined detection techniques, we changed the periodicity of testing from quarterly to triennially, further costs of both animals and testing. Additionally, the latest technology allows for pooled testing of individual dried blood samples and pooled fecal pellets for parasite testing representative of the room. Individual testing of a pooled sample occurs if it tests positive for a pathogen, thus there is no threat to vivaria biosecurity. This resulted in dramatic reduction of costs for testing by nearly **50%** yet without compromising vivaria security.

PD9

Comparison of ventilated rack Exhaust Air Dust to soiled bedding sentinels for detecting mouse pathogens

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Abstract
Sampling of accumulated debris within the exhaust system of ventilated rodent racks can be used to survey rodent colonies for infectious agents. This study examined the effectiveness of pathogen detection by PCR analysis of exhaust debris collected from Tecniplast and Allentown racks fitted with Interceptor (n=1) or Sentinel™ media (n=1), respectively, to that of sentinel mice receiving soiled bedding (n=10 cages). For 12 weeks, racks were populated with 5 cages of mice (3 mice per cage) infected with one of the following pathogens: mouse norovirus (MNV), mouse parvovirus (MPV), mouse hepatitis virus (MHV), *Helicobacter* spp., *Pasteurella pneumotropica*, pinworms, *Entamoeba muris*, *Tritrichomonas muris*, and fur mites. Soiled bedding was transferred to sentinel mouse cages every 2 weeks and pathogen shedding by infected mice was monitored throughout the study. Pathogens testing positive by PCR analysis of exhaust debris included MNV, MPV, MHV, *Helicobacter* spp., *P. pneumotropica*, *Entamoeba muris*, pinworms, and fur mites; *Tritrichomonas muris* was not detected in exhaust debris from either rack. All pathogens were detected in at least 1 cage of sentinel mice by serologic evaluation for antibodies or PCR testing of feces or fur swabs except for *Pasteurella pneumotropica*, *Tritrichomonas muris* and fur mites. These results demonstrate that under these conditions, PCR testing of rack exhaust debris detected more infectious agents than samples from mice receiving soiled bedding and is a viable alternative to soiled bedding sentinels to monitor mouse colonies for infectious agents.

PD10

Toward accurate detection of opportunistic mouse pathogens

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Abstract
[Background] RIKEN BRC has been engaged in the collection, preservation, quality control, and distribution of experimental mice. As many of the deposited mice have unwanted contaminants, extensive microbiological monitoring is carried out after rederivation. Bacteriological monitoring by culture needs trained technicians and continuous education is required. We have started genome sequence collection of mouse monitoring organisms focusing on protozoa and opportunistic bacteria, and our goal is development of accurate PCR tests targeting these organisms. Here we present our recent progress of bacterial results. [Methods] PacBio RSII and Illumina HiSeq 2500 were used to sequence genomic DNA. HiSeq data were mapped to PacBio data to correct sequence error and complete genome sequences. [Results and Discussion] We have almost finished genome sequencing as follows: 2,691,209 bp from *Rodentibacter heylii*ATCC 12555^T, 2,322,975 bp from *R. pneumotropicus*JCM 14074^{T}and 2,600,928 bp from *Corynebacterium bovis* JCM 11947^T. JCM 14074 and JCM 11947 also had small circular DNA (30 – 50 kbp each). Our tentative analyses show that clinical isolates obtained from mice contaminated with these pathogens have genetic polymorphism in not only 16S rRNA operons but also in several house-keeping genes. Based on our entire genome sequences as the references, precise genomic structure of these bacteria will be clarified by the detailed analyses, which is useful to find new PCR targets. Furthermore complete genome information will help us to know pathogenic mechanisms of these bacteria.

PD11

Comparing dirty bedding and Exhaust Air Dust sentinel program during year-round routine health monitoring

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Abstract
Health monitoring in rodent facilities is a challenging duty. Most frequently soiled bedding sentinels are used. Recently, exhaust air dust control (EAD) emerged as a new monitoring method by using a sensitive and specific real-time PCR assay. We performed a year-round testing of the EAD monitoring in parallel to the existing regular bedding sentinel program on a quarterly interval in all of our rodent units with different health status level. Additionally, we tested four different providers of EAD analyses as we equipped all our IVC filter towers with double testing material. During the year-round routine health controls, monitoring by EAD was able to detect a range of pathogens like Helicobacter spp., Pasteurella pneumotropica, Astrovirus, Norovirus, MPV and Pinworms. Some of these agents were not detected with the routine bedding sentinel program including Pinworm: Only one cage in an experimental unit was contaminated with Syphacia obvelata due to an import. One positive cage out of 280 cages led to a positive EAD result whereas regular bedding sentinel setting (one sentinel cage for 140 cages) was not able to detect this Pinworm infestation. Additional testing was undertaken like PCR analyses of fecal pellets and direct microscopy after fecal flotation and confirmed infestation of only one cage with imported mice. The MPV infection was revealed by EAD as well as by the routine bedding sentinel program. EAD delivers reliable results. Close communication with the providers of the different analyses and monitoring methods is crucial for decision making especially in case of unexpected findings.

PD12

Comparative study of Exhaust Air Dust testing and traditional bedding sentinels for health monitoring

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Abstract
Alternative health monitoring such as Exhaust Air Dust (EAD®) PCR testing is a new method designed to improve the detection of pathogens. EAD® can be easily collected with an in-line capture media available in different IVC rack systems.

Three independent research groups performed a health monitoring comparison study. They compared the accuracy of environmental health monitoring by using an in line EAD filter in IVC racks (N=26) and the traditional bedding sentinel methodology.

Test specimens at 1, 2 and 3 months included prefilter swabs (N=7) and in line EAD filters (N=15) for environmental samples. Each sample type was pre-processed and DNA/RNA were extracted using a total nucleic acid isolation kit. Sample nucleic acid was screened by individual single-plex PCR assays on a real time open array PCR platform for over 55 infectious agents. Semi quantification was performed to define copy number per reaction. At 3 months, sentinels (N=26) were sent to laboratories for traditional testing including bacteriology, serology, and parasitology.

The data showed a significant increase in detection rate with the filter media. In overall, the average percentage of detected agents using the media filter was 7 time higher as compared to the sentinels. Most of the agents were detected after 1 month of exposure (*Helicobacter spp*, MNV, *P. pneumotropica*). However the opportunistic agents such as *S. aureus, K. oxytoca, P. aeruginosa* showed a delayed detection at 2 or 3 months. The benefits of EAD testing include an improvement of detection and a reduction of animals used.

PD13

Health monitoring in a rodent facility: Implementation of the Exhaust Air Dust system

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Abstract
For years, sentinel animals have been considered as the reference for health monitoring. In 2017, the newly available Exhaust Air Dust system (EAD, Interceptor Tecniplast®) was implemented in the EPFL rodents facilities. A comparison of the results obtained with the EADs with those obtained with sentinels was done. The aim was to determine if this new technology was as reliable as the usual methodology and would allow reducing the number of animals dedicated to health monitoring.

When comparing the results over two years, it appears that:

1. EADs filters allowed for a more reliable detection of given low contagious microbiological agents hardly detected by the classical sentinel technique (*e.g.*, *Pasteurella pneumotropica*),
2. The results were comparable for the agents usually present in the animal facilities.

In 2019, EADs have been implemented in the health monitoring strategy for all the facilities, in parallel to the use of sentinels. We expect with this EAD technique a significant decrease in the annual number of dedicated animals by up to 60%.

Some questions remain open regarding this new system. The parallel use of live sentinels could allow an earlier detection of disease outbreaks by clinical observation, while EADs only detect the presence or absence of the causing agent(s). Furthermore, EADs necessarily imply the use of PCR, which has a very high sensitivity but also some limitations, like false positives. Currently, only limited information is available about the efficiency of EADs to detect rarer agents with significant effects on both animal health and research outcomes.

PD14

Results of classical versus PCR health monitoring in three species and different diagnostic labs

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Abstract
During recent years PCR methodology has been increasingly used for routine health monitoring of lab animal colonies. However, the practicality, reliability and reproducibility are still under discussion. In this study we analyzed the health status of mice, rats and zebrafish by means of classical methods like serology, bacteriology and parasitology and PCR technology in the same animals and by environmental sampling. All rodent and zebrafish samples were analyzed in three external commercial diagnostic labs according to annual FELASA, respectively ZIRC profile. Results were compared in terms of diagnostic outcome, time from shipping out the samples to the receipt of the results, animal welfare and 3 R's and costs with special consideration to sample pooling and the possibility of environmental monitoring. All pathogens detected by classical methods were also found by PCR health monitoring (PCRHM). By PCR techniques more agents could be detected in comparison to classical methods. Analysis of the same samples may not lead to identical results in different diagnostic labs. In conclusion health monitoring by PCR is superior compared to classical health monitoring methods. PCRHM improves the detection rate of pathogens and exhibits a higher sensitivity. Results are usually received faster, which allows faster decision making in the animal facility. In terms of animal welfare PCRHM offers significant advantages since sample collection is non-invasive and less stressful for the animal. Sampling

does not require killing or shipping of live animals. Using sample pooling and environmental monitoring significant cost savings can be achieved.

PD15

Tackling differences in health monitoring among italian animal facilities

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Abstract
Defining health status of animal facilities is important not only for importing/exporting animals, but also for research reproducibility. As already shown by PREPARE guidelines, health monitoring (HM) is one of the parameters affecting quality of the study. However, different facilities apply different methods (sentinels, sampling animals of the colony, environmental monitoring) and type of protocols (pathogens and number of animals tested for). Proper information on health status of animals housed in the facility help researchers to properly design experiments: unknown infections and infestations affect data, that can be misinterpreted and, on the long run, being not reproducible. During September 2018, we run an anonimous survey (16 questions in total on Survey Monkey platform) among AISAL (Italian Association of Lab Animal Science) members regarding housing systems and HM of their facility. The survey was intended to identify i) how HM is performed (animals and microbiological agents tested, frequency, *Helicobacter* spp. identification, screening of biological monitoring; ii) if there is any correlation between capacity of facility, HM methodology and research fields; iii) how HM results are shared with researchers; iv) critical bottlenecks in standardization of housing, HM and data reproducibility. We got 20 responses, so these results are not representative of the Italian situation, but still it can provide inputs on which limitations veterinarians, facility managers and researchers are facing when dealing with health monitoring. Further more, we aim to run another survey with a Delphi approach, to address more specifically some of the limitations of the first survey.

PD16

Efficacy of HClO spray system in animal facilities

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Abstract
Hypochlorous acid (HClO) is a weak acid and produced by neutrophil in our body, in order to destroy invaded bacteria. It is widely used as disinfectant in the cosmetics industry, water and food preparations. Our facility has installed a HClO spray system recently. This study is aim to test the efficacy of disinfection of HClO spray, and find out what frequency and spraying period provides sufficient disinfection effect. We prepared equal concentration of *Staphylococcus xylosus* suspension 100 µl and swabbed on blood agar plates for tests We tested various frequency of HClO spray: 10 sec/2 hrs., 15 sec/1 hr., 30 sec/0.5 hr., 60 sec/0.5 hr., 90 sec/0.5 hr. and. 60 sec/0.25 hr. All plates were placed in a sealed and HClO fogged room. While the tested group exposed to the HClO fog, the control group was covered to prevent the exposure to HClO fog. Both plates were placed in the HClO fogged room for 24 hours, then removed to the $21 \pm 2^{\circ}$C incubator. After 72 hours' incubation, the plates were observed and measured the number and area of colonies by a computer software. We found that the number of colonies decreased, but not consistent. However, the total area of colonies decreased prominently. It decreased from 22.7% up to 86.4%. It indicated that the HClO fog could inhibit the proliferation of bacterial colonies obviously. We concluded that HClO spray system could be a useful tool to reduce bacteria in the animal facility.

PD17

Environmental enrichment program for rodents in a ABSL3 unit

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Abstract
Environmental enrichment (EE) refers to modifications in the environment of captive animals that enhance their well-being by providing stimuli which meet their species-specific needs. European regulations require that all animal research centres have up-to-date EE programmes. In our ABSL3 research centre, rodents are kept for research on new treatments for infectious diseases, and a programme of EE has been in place since 2006. In this communication we describe our current programme with examples for several mouse and rat strains. The specific characteristics of ABSL3 facilities, in addition to working with immunodeficient mice, make the choice of EE particularly challenging. Work with infectious agents and immunodeficient mice means all items need to be sterile in & out. Work on air-transmitted diseases is done in half-suit isolators with a three-glove system. This complicates animal handling and procedures, so enrichments selected should not be a further obstacle. Additionally, for mice, our programme has been tailored not only to species, but also to strain. For example, default enrichment for mice is nesting material and a shelter. However, BALB/c build nests with a more complex structure than other strains but do not use plastic shelters. Therefore, two types of nesting are provided instead of shelters. BALB/c build now very complex nests, with the more rigid material used for structure and the softer material used to line the inside of the nest. EE programmes need to be updated regularly to keep up with new findings and to adapt to the needs of animals and research.

PD18

A new bedding for marmosets enhances animal welfare and staff's wellbeing

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Abstract
The common marmoset (*Calltihrix jacchus*) is one the major non human primate species used in biomedical research. A colony of 130 animals is currently housed at Silabe-University of Strasbourg in pens or cages. Sawdust is distributed to encourage foraging but cleaning in cages represent tough work for staff. We tested a new bedding called wood wool pad, made of kraft paper and glued wood fibers. This way, bedding can be directly put on the grids of the cages whereas sawdust had to be spread on a tray. Cleaning process is therefore twice reduced and less arduous because the bottom of the cage has not to be removed anymore. Moreover, cleaning frequency could also be twice reduced thanks to a better durability. Last but not least, a behavioral study has been conducted to validate the new bedding as foraging material. Eight family groups of around six individuals housed in cages have been alternatively observed on sawdust and on wood wool pads. Each bedding has been implemented for seven days and behaviors of each group have been observed live by 30-minutes focal sampling on Day-1, D-2, D-6 and D-7, twice in one month. The results have shown that marmosets spent more than twice of time on wood wool pads than on sawdust. Besides, the new bedding promoted more desired behaviors such as manipulation and affiliative interactions. To resume, the wood wool pad has been validated as bedding material, improving not only animal welfare but also staff's wellbeing.

PD19

Why not Porcichew for macaques?

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Abstract
Environmental enrichment is part of the refinement process required and engaged by centers hosting non-human primates (NHPs). Knowing that the perfect enrichment does not exist, finding the most suitable device involves efforts to look at some permitting to improve animal welfare as well as being adapted to husbandry constraints. Macaques are the most NHP species used in biomedical research and also the most challenging regarding suitable enrichments. Indeed, devices should be safe, resistant, long-term attractive and easily cleanable. A product which complies with these criteria is currently used for pigs and will be supplied by PLEXX B.V, the Porcichew. Aiming to reduce tail-biting, the device is made of biodegradable malleable material and impregnated with strong appealing scents which attract the pig the more it nibbles it. The material composition is anti-bacterial and could therefore be distributed in confined areas in which other material like wood cannot be used. All these properties make the Porcichew a very interesting device and could be potentially beneficial for NHPs for which no equivalent exists. In this purpose, this study wants to investigate the effectiveness of the Porcichew on NHPs. The device of different flavours (apple, vanilla, aniseed, chocolate, truffle) will be tested in April 2019 on macaques. We hypothetise that compared to a new malleable enrichment without scent, the Porcichew will be more used along time due to the scent's releasing property. Results will be presented in the poster with the aim of presenting recommendations to optimize husbandry and culture of care of NHPs.

PD20

Stiff as a board: Measuring rigor mortis in zebrafish

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Abstract
According to Schedule 1 in the Animals (Scientific Procedures) Act 1986, euthanasia must include both a humane method of death and a confirmation of death. For zebrafish, anaesthetic overdose is the appropriate method of death; however, there is widespread variation on which anaesthetic agent is used. Confirmation of death for zebrafish is typically one of three options: destruction of the brain, instantaneous destruction in a macerator, and confirmation of rigor mortis. The latter method can be the simplest, but not necessarily the quickest; however, all of these methods can frustrate any potential post-mortem work that may be required. Additionally, anecdotal evidence suggests that different anaesthetics can inhibit or reduce the rate of the onset of rigor mortis, with some taking as little as an hour, whilst others can take more than three. The mechanism behind the reduction of rigor mortis rates with the different anaesthetic agents is not fully understood, although there is a potential relationship between the effects of anaesthesia on oxygen or potentially pH within the tissue. In order to determine the most time effective method of confirmation of rigor mortis for fish euthanised via an anaesthetic overdose, trials were conducted testing multiple anaesthetic agents on two non-sacrificial wild type strains. These were then monitored in timed intervals in order to identify the rate of rigor mortis for the different anaesthetics. The resulting preliminary index of rigor mortis rates with these agents can aid in the decision making process for anaesthetic overdose in S1K euthanasia for post-mortem work.

PD21

Comparison of zebrafish embryo collection using various methods

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Abstract
With the increase of scientific need for high volume output of good quality embryos from zebrafish has also come the rise of various breeding strategies and the development and modification of tools

in order to fulfill this need. And whilst much work has gone into this, the welfare of both parent and progeny may come second to the procurement of the desired embryo numbers. In order to determine if there is such a cost, a comparison was made between five different spawning methods and the quality of embryos that each produced: pairing, trays, and two types of mass embryo collection units, one of which was modified to become a preference choice based unit. These different methods and tools produced a variation of embryo volume as well as quality, indicating that stress has a negative impact on embryo viability. From both a scientific and welfare perspective, good breeding strategies are key; they should reduce stress, and allow fish to express mate preference, as well as to choose whether to spawn or not. This type of refinement can aid in devising breeding strategies that can use fewer numbers of fish and still produce a high volume of embryos.

PD22

Introduction of a Step-by-Step Protocol for the eradication of Mycobacterium haemophilum in zebrafish system

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Abstract
In 2017, the zebrafish unit at University of Glasgow experienced a detrimental outbreak of pathogenic bacterium, *Mycobacterium haemophilum*. The presence of other bacterial species was also confirmed by bacteriology growth. The affected individuals composed of a wild-origin parental population sourced from India and their F1 offspring generation (in house). Bacteria were diagnostically confirmed to be present systemically in fish and within the water and biofilm of the recirculating zebrafish systems. In the absence of a publicly accessible step-by-step disinfectant protocol for these difficult-to-eliminate pathogens, we devised a successful procedure to eradicate *M. haemophilum* and Aeromonas species after colony removal using chlorine tablets (active ingredient Sodium dichloroisocyanurate) and Virkon Aquatic. Postdisinfection diagnostics did not detect pathogens in the system or in the new fish inhabiting the system that were tested. Newly established fish colonies have not shown similar clinical signs or disease-induced mortality in the 1-year period following system disinfection and repopulation. Our aim is to provide a detailed disinfection procedure for the effective elimination of *M. haemophilum* and *A. hydrophila* from research-standard zebrafish units.

The simplicity of this disinfection protocol allows for simple adjustment to be used in different settings, such as flow-through or recirculation systems (both glass and polycarbonate designs), and can be adapted to cater to smaller or larger scales for other aquatic facilities as well. It is a cost and time effective method to use for facility or quarantine unit disinfection before the introduction of new colonies of fish.

PD23

Anaesthetic efficacy and aversion: Comparing 2 common zebrafish protocols with a combination of propofol/lidocaine

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Abstract
Zebrafish as a laboratory animal model has been continuously rising in interest. This evolution in usage asks for more refined protocols such as in anaesthesia, one of the most commonly used procedures. Thus, we aim to study the clinical efficacy of as well as fish aversion to equipotent dose of propofol/lidocaine in combination, MS222 and clove oil. After determination of the equipotent doses, i.e. concentrations that induced the loss of equilibrium at similar times, 54 mixed-sex adult AB zebrafish were randomly assigned to MS222 (150 mg.L-1), Propofol/lidocaine (5mg.L-1 propofol + 150 mg.L-1 lidocaine) and Clove Oil (45 mg.L-1) group. Latency to lose response to a painful stimulus and to recover were assessed. To study aversion to these anaesthetic protocols, the conditioned place aversion test was used, where aversion was measured by the latency to go to the conditioned place, where animals previously experienced the anaesthetic. HCl (pH = 3) (n = 8) was used as the positive control.

Clinically, MS222-treated animals recovered faster averaging 90 seconds ($p < 0.012$) and Clove oil-treated animals took longer to lose pain response averaging 484 seconds ($p < 0.002$). Our preliminary results from the conditioned aversion test showed no marked aversion related to any anaesthetic when compared with the positive control, but the study will be extended. Based on our present data, all protocols seem to be acceptable for adult zebrafish, but more research is ongoing to clarify potential side-effects regarding physiological, cellular and behavioural alterations.

PD24

Standardization of zebrafish housing systems. Next destination: metals and metalloids

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Abstract
The metals and metalloids represent most of the chemical elements found in Earth. Well-described *essential metals* are divided into macronutrients (Ca, Mg, Na and K) and micronutrients (Cu, Zn, Fe, Co, Mo, Cr and Mn). Macronutrients are relevant for general physiological processes and micronutrient play a role in certain biological processes a low concentrations, acting as cofactors or integral parts of enzymes. *Non-essential* metals are poorly described in general, few of them are known to have toxic effect (like Pb, Cd, Hg, Al), or to be required for certain species (as V, Rb, Ni and Ge) with no clear mechanisms of their action. For the rest of the metals, there is not enough data to be certain about their requirement or toxicity for organisms. Governmental agencies report acute or chronic aquatic water criteria for some metals, but in most of the cases, there are no clear descriptions or enough information on safe vs toxic water concentrations. For this reason, in collaboration with other facilities, we characterized the concentration of metals and metalloids found in *Danio rerio* systems, with the aim to better understand the origin of these elements, to improve the reproducibility of research, and to contribute to the welfare of this widely used animal model.

PD25

A Semi-automatic dispenser, RFID-based system, for controlled liquid or solid zebrafish feeding

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Abstract
We develop a novel, low-footprint and low-cost semi-automatic system for delivering solid and liquid food to zebrafish, and more generally to aquatic animals raised in racks of tanks. It is composed of a portable main module equipped with a contactless reader that adjusts the quantity to deliver for each tank, and either a solid food module or a liquid food module. Solid food comprises virtually any kind of dry powder or grains below two millimeters in diameter, and, for liquid-mediated food, brine shrimps (*Artemia salina*) and rotifers (*Rotifera*) have been successfully tested. Real-world testing, feedback and validation have been performed in a zebrafish facility for several months. In comparison with manual feeding this system mitigates the appearance of musculoskeletal disorders among regularly-feeding staff, and let operators observe the animals' behavior instead of being focused on quantities to deliver. We also tested the accuracy of both humans and our dispenser and found that the semi-automatic system is much more reliable, with respectively 7-fold and 84-fold drops in standard deviation for solid and liquid food.

PD27: Withdrawn

PD28: Withdrawn

PD29

The naked mole rat as a model for research – Improving its welfare under captivity

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Abstract
The naked mole rat (*Heterocephalus Glaber*) has gained importance as a biomedical research model for various conditions like hypoxic brain injury, cancer, ageing, and nociception. Much is left to discover how this species best should be housed for experimental purposes. Determining effects of light/dark cycles, feeding and behaviour as well as establishing a biomarker of stress, could help scientists towards improving welfare in the NMR under captivity. A total of 56 naked mole rats were housed either under 24 hour darkness; 24 hour light; or 12/12 hour light: darkness-cycles. Light:darkness-cycle did not significantly affect colony feed consumption and subsequent weights. The behaviours observed in the animals were huddling; patrolling/ exploring the cage; feeding, licking paws, tail or anus; climbing walls of the cage; and carrying of objects in the mouth. Only huddling behavior was significantly different ($P < 0.05$) between animals housed in 24-hour light versus 24-hour darkness. To establish corticosteroids as a biomarker for stress, serum samples were tested for corticosterone and cortisol after ACTH injection compared to control (saline injection). The corticosterone concentration was significantly higher ($p < 0.05$) after ACTH injection (90.30 ± 7.791 nmol/L) compared to controls (24.13 ± 4.343 nmol/L). The cortisol concentration was 1.13 ± 0.187 ng/ml after ACTH injection, whilst undetectable in the control group. The results indicate that light: darkness-cycle doesn't immediately affect the behavioral patterns studied, and that corticosterone may be considered the preferred stress biomarker in the naked mole rats.

PD30

Rabbit welfare assessment (laboratory and meat purposes): Enclosures with plastic floors: Holes versus slats

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Abstract
Due to welfare concerns and legal restrictions in certain countries, alternatives to wire net floors must be developed in rabbit husbandries. Also, there is a difference in regulations in Europe for laboratory rabbits vs. rabbits bred and kept for meat production. While there are regulations concerning floor design of enclosures for rabbits bred for meat production in many European countries, the European Directive 2010/63 lacks regulations for rabbits used for scientific purposes. This study compares two floors, which meet the Austrian legal requirements for growing rabbits intended for consumption as well as the requirements for laboratory rabbits. The dual use of rabbits bred for meat production and applicable for scientific purposes would avoid the problem of surplus animals of specialized producers for laboratory rabbits.

A noryl floor with 12mm circular holes was compared to a 10mm slatted plastic floor. Parameters were soiling of cages and animals, parasitic burden, clinical health, and losses using objective scoring. Soiling of cages and animals and coccidial oocytes were significantly higher on the floors with circular holes. Obvious signs of disease showed a non-significant trend to be more frequent in the group with circular holes. This was linked with significantly higher losses. In conclusion, our study clearly shows that the floor with circular hole design cannot be endorsed, although it meets legal requirements. The slatted floor type can be cautiously recommended; however, to assure animal welfare in laboratory rabbits, legal authorities in Europe should take on the responsibility of regulating floor design in this sector.

PD31

Actualities in the chinchilla (Chinchilla sp.) healthcare

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Abstract
As a veterinarian, specialized in domesticated chinchillas (*Chinchilla sp.*), the author carries out veterinary and expert tasks at chinchilla farms in 12 countries worldwide, supervised over 60.000 animals. The aim of the presentation is to describe shortly some of the most common diseases of chinchillas in the breeding and research facilities, looking first at maintenance defects, parasitoses, bacterial infections and the possibilities to protect against them.

The following animal health problems will be presented in the lecture in the rule of causes, external examination and pathological findings, diagnostic procedures, prevention and treatment:

-Caregiver's errors
-Interspecific agression
-Heat schock, heat tolerance
-Shortcomings in feed composition
-Fur chewing, Fur loss, *Trichophyton* mentagrophytes, Cheyletiella parasitovorax
-*Giardia duodenalis A, B, C, D*
-*Spironucleus muris, Trichomonas muris, Cryptosporidium ssp.*
-*Escherichia coli, Proteus mirabilis, Streptococcus hyointestinalis, Clostridium sordellii*
-*Listeria monocytogenes, Salmonella typhimurium*
-*Streptococcus canis*

PE1

Recognising animal abuse and assessing its extent

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Abstract
The importance of implementing my project extends to working with animals. To provide animal's wellbeing plays a human ethical role, especially, if they kept in closed conditions or they belong to species, which are unviable without human care. Animal testing is a critical area of the animal protection, because the animal's wellbeing is unavoidable during certain examinations, furthermore the protection of the test animal's welfare is the basic of the successful experiments.

Joining the research on animal protection, my aim was to make assessment of animal abuse and the wellbeing of animals measurable. I consulted with people who are playing an active role in judging cases of animal abuse. Based on the responses I made an online reporting interface, which could be life-saving for animals, thus it could be a significant step to be introduced in daily life.

The next step it would be important to extend the interface, while it could help to allow the observation of the laboratory animals in the animal testing institues. It could facilitate the work of the persons checking the animal house, it also gives possibility to strain off the forbidden or irregular made attempts, and this document can be able to use during the investigation of the cases in question. On the registration form would come into view the continually traceableness of the enforcement of ethical- and 3R precepts. As a result, it can frame a checklist, which could help in the control of animal testing in all European Union member states.

PE2

Improving animal welfare and staff care through maximising learning from observations and events

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Abstract
The team initiated an 'Observation and Event Learning Log'' a systematic framework to capture everything with potential to impact animal welfare positively or negatively including:

- Visible process of recognition (including caring approaches e.g. recognising a member of staff for a particularly caring approach to handling animals upon receipt)
- Embedding root cause identification; actions to prevent reoccurrence and open sharing of learning
- Periodic review identifying trends of minor observations thus improving animal care and supporting technical and science staff
- Transparency – Open AWERB meetings share learning and recognition supporting a culture of challenge and care

One indicator of a supportive culture is willingness to openly share success and areas for improvement. Between Jan-17 and December-18 over 250 observations were logged and reported through four Animal Welfare and Ethical Review Body reports. We created an environment of continuous improvement for both animals and staff by recognising, rewarding and sharing good practice and where near misses and incidents are openly reported, investigated and learnt from.

Example of impacts:

- Staff registered a low incidence of scabs associated with the administration of Carprofen as a post-surgery analgesic; the trend was fully investigated. Discussion at AWERB based on Harm Benefit Analysis allayed staff concerns.
- A systematic analysis of events relating to checking errors versus workload. We continued to support and promote staff diligence, but also supported staff by showing with data how we need to better manage peaks in workloads.

PE3

The role and limitations of prospective project evaluation in animal research

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Abstract
Directive 2010/63/EU mandates in EU member states that project proposals involving procedures on living non-human vertebrates and cephalopods require authorization in a review process that includes a Harm-Benefit-Analysis, which assesses ''whether the harm to the animals in terms of suffering, pain and distress is justified by the expected outcome taking into account ethical consideration and may ultimately benefit human beings, animals or the environment.

The aim of this talk is i) to summarize recent criticism on the epistemic and practical limitations of prospective benefit assessment in the HBA and ii) to discuss the role and limitations of ''ethical considerations'' of benefits within the boundaries of the normative framework of the Directive.

First, we demonstrate that prospective evaluation of practical benefit suffers from a logical and methodological flaw. The outcome of an experiment is per definition uncertain. If it wasn't, the experiment would not generate new knowledge and would therefore be illegal. Moreover, there will always be uncertainty regarding the translatability of knowledge from animal model to target species. Second, we discuss practical flaws which further exacerbate the problem of evaluating practical benefit prospectively, e.g. non-scientific factors, such as market potential, lobbying, patient compliance, etc., are impossible to predict and yet, are important parameters in prospective benefit assessment. Together, these uncertainties make prospective assessment of practical benefits implausible.

Last, project evaluations are legally required to comply with the normative framework of the Directive. Against this background we discuss the role and limitations of ''ethical considerations'' of benefit.

PE4

It's time to rethink: Critical Incident Reporting System in Laboratory Animal Science (CIRS-LAS.de)

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Abstract
From today's perspective and considering the current state of medicine, use of animals in experimental purposes cannot be completely dispensed. A large number of scientific articles based on animal experimental studies are published. But negative experiences gained from these experiments get lost or are not referred in publications. The objective of the CIRS-LAS portal is the detection of critical incidents in the entire range of laboratory animal science. Thus, an open dialogue of failures can help to avoid them in the future. CIRS-LAS is the world's first published CIRS in laboratory animal science and plays an exemplary pioneering role in the implementation of the 3R principles reduce and refine: reduce the number of laboratory animals and improve animal safety.

The CIRS-LAS portal is based on similar databases in human medicine and allows anonymous reports of critical incidents. Currently, more than 70 people from Europe are already registered users of the CIRS-LAS portal and more than 50 critical incidents have already been entered. Registered persons can comment and discuss the entered critical incidents to share their experiences.

In conclusion, the realization of 3R can be achieved by using the world's first published CIRS-LAS portal. Anonymously shared negative experiences from animal experiments can influence the experimental setup of animal based research. Furthermore, it is conceivable that the implementation of CIRS-LAS serves to enhance the trust in laboratory animal science of both, public and scientific community. The time to rethink has been achieved – to learn from negative results in animal based research!

PE5

Challenging harms in the Harm-Benefit-Analysis: The case of harm assessment in genetically altered animals

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Abstract
The use of animals in research requires careful ethical consideration of whether the burden on the animals is justified. As one important part of the project evaluation, a harm-benefit-analysis (HBA) has to be carried out in order to approve projects in line with EU Directive 2010/63/EU. This implies that harms and benefits of a particular project have to be assessed prospectively beforehand to weigh them afterwards. Although there are different models of weighing, it is clear that an assessment of prospective harms and benefits is a precondition for any weighing procedure. In this context, projects for the generation of new genetically altered (GA) lines or breeding and maintenance of GA animals that are likely to develop a harmful phenotype raise new issues. The unique feature of new GA lines is that a significant lack of knowledge makes it difficult and logically impossible to estimate harms prospectively with sufficient certainty since it is not predictable what sort of harm – if at all – the animals are going to experience. Therefore, this contribution aims at dealing with the new challenges of harm assessment in GA animals and its implications on the HBA. A practical guideline will be presented that serves as an overview and guidance for relevant harm factors and addresses the main challenges, e.g. dealing with uncertainties in the process of HBA.

PE6

How can competent authorities contribute to better animal welfare in science?

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Abstract
To highlight the 3Rs, the Norwegian competent authority focus on dialog with establishments and researchers.: Good animal welfare in science is not about a well-formulated project, it is about well-performed experiments.

1. **Cooperate with the establishments and the animal welfare bodies!**

Make use of the local sense of responsibility and legal demands. We all seek to achieve a common goal; To lower the cost for the animal and increase the benefit of the research. Be in dialogue, be constructive and competent.

Focus on the requirements and the tasks of the personnel in the establishments. The applications for experiments can be pre-evaluated locally. This will strengthen the establishment's authority. Also, our decisions strengthen the establishments: *''Should any disagreement arise regarding humane endpoints or termination of a procedure, the person responsible for the welfare and care of animals and the animal-welfare body shall decide''.*

1. **Cooperate with the researchers!**

Before granting project authorization, challenge the applicant in a competent and respectful way. Require ''to-the-point''-information.

If not well described in the application, we ask about the expected outcomes, which methods/procedures will be performed, which methods have been rejected and why, which alternative methods have been considered, challenge the design, challenge the justification of the experiment etc. The experiment should give relevant answers to well formulated biological questions.

For about half of the applications or more we exchange emails or make a call to the applicant. We have contributed to refined analgesia and anesthesia protocols, better humane endpoints and refined procedures.

PE7

Ethics committee regulations animal experiments in Turkey

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Abstract
In Turkey, the first regulations on animal experimentation ethics committees was published in 2006. This regulation was revised in 2014 and detailed in line with the EU Directives 2010/63/EU.

In the revised regulation; in order to evaluate the applications to be made, the mandatory information on the form to be prepared is presented in detail. In the experiments, anesthesia applications, methods of killing animals and classification of violence in the experiments, re-use of animals in experiments and the conditions for termination of the experiment are indicated.

The evaluation criteria of the projects were determined in the regulation and detailed information was given about the training of the staff dealing with the experimental animal. As of November 30, 2017, training programs have been made compulsory for animal species. In parallel, studies on informing educators about species-specific education have been initiated and are still ongoing.

PE8

The current R concepts to increase awareness: Preliminary study

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Abstract
There are national and international legal initiatives that govern the use of animals in research in Turkey. 3-R principle directs scientists about the ethical use of animals in scientific studies. In addition to the basic principles of 3-R, in order to increase awareness in legal and social regulations, the new R concepts are added as a lessons for training courses. Rehabilitation, Responsibility, Respect, Reuse, and Review are recent R concepts. In addition as a lesson, responsibilities are increased with current R concepts. Changes in Turkey in the last period covered by EU Directives in compliance framework is made and steps taken to ensure compliance. Although increases in parallel with legislative work on the developments in Turkey, 3Rs principle, which is the cornerstone of experimental studies; while it is considered as a research culture in many countries, it is seen as a necessity in the legal framework in our country. In addition to the basic principles of experimental studies, the current R concepts have been started to be included in the courses. On preliminary study we see that 80% of the researchers reported that their ability to empathize increased and become more sensitive for animal welfare. According to the Preliminary study we have been started to be included in the regulations in order to put the current R concepts as a research principle as well as the legal requirements as well as a cultural accumulation.

PE9

Challenges encountered in implementation of IACUC role in developing countries

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Abstract
Although the role of the IACUC is well established in developed countries and the laws that regulate their operation are under continuous review and updates, in developing countries the newly implemented IACUCs are encountering many obstacles and difficulties. At start of the millennium in Egypt, research ethics committees in the different academic institutions aimed to enhance the research activities of both human and nonhuman animals in an ethical framework. In 2013, Cairo University established first IACUC in Egypt in accordance to OIEmandate and follows international guidelines in its review of animal protocols. Since then it has set an action plan to increase awareness of animal welfare principles with emphasis on the 3Rs, provide training resources to the researchers, design standard of operational procedure for its committee, and develop national guidelines. Training and education of researchers were integrated into the postgraduate educational program and in training program for faculty members' development. Egyptian constitution and regulations of protocol submission as required by the university were among the opportunities that foster the development of animal care and use in research and education. Based on data collected over the last year some challenges that face the IACUC were elucidated. Dilemma of sample size calculation and the principle of reduction, the need to consider replacement before formulating a research question and designing a research plan, the concept of randomization and risk of bias, as well as pain relief in studies where analgesics may affect the outcome of the study are some of the difficulties encountered.

PE10

The Norecopa database: A collection of global 3R resources

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Abstract
The Norwegian 3R Centre Norecopa maintains a website with, currently, over 7,500 pages of resources for implementing the 3Rs (https://norecopa.no). The website was totally redesigned in 2016, using the latest technology, to make it easy to use on all

platforms and incorporating an intelligent search engine which has indexed all the words on the site.

Among the contents of the website are:

1. A global overview of guidelines, 3R centres, journals and email lists
2. Information on over 3,000 educational and training aids which may be used as alternatives or supplements to animal use
3. Information on over 1,700 textbooks and other literature within Laboratory Animal Science and related fields

The search engine and a large number of search filters can be used to increase or narrow the results of a search easily.

The website also houses the PREPARE guidelines for planning animal research and testing (https://norecopa.no/PREPARE), an International Meetings Calendar, and a closed discussion forum for Norwegian laboratory animal specialists. Furthermore, Norecopa's website is the domain of the International Culture of Care Network (https://norecopa.no/more-resources/culture-of-care).

The Norecopa website is updated many times a week, and aims to be a one-stop-shop for links to resources within all aspects of the 3Rs. Norecopa also issues an English-language newsletter 7-8 times a year, which highlights major additions to the website. The website attracts at present approximately 180,000 pageviews per year.

A 17-minute video presentation of the Norecopa website is available at https://norecopa.no/info.

PE11

Implementation of a vivarium management software: a retrospective view

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Abstract

The Central Animal Facility of Hannover Medical School (Ztm) has capacity to maintain approximately 80,000 animals (rodents, rabbits, sheep, pigs, zebrafish). Currently more than 1000 different mouse strains are bred within different hygienic barriers. This includes a huge number of genetically-modified strains some of which show a harmful phenotype. Given this complexity, we aimed to implement a management software to ensure compliance with the legal framework for documentation and reporting (EU-Directive-2010/63, German law on genetic engineering and German-VersuchstiermeldeVO). In addition, we intended to improve communication between researchers and animal caretakers, to reduce paper print-outs and to facilitate access to information on ongoing animal studies. In 2017, the decision was made to introduce LAVAN. In the first phase, LAVAN was rolled out in the biggest SPF breeding barrier (5000 cages). By the beginning of 2018, the whole implementation was completed including the incorporation of strain information and animal licenses. The use of LAVAN implied major changes. Cages are now only labeled by position numbers, leading to an obligatory use of tablet computers for all people in the animal rooms. Researchers can do breeding management and experimental planning from their desktop without physical access the animal rooms. Furthermore, there is now a comprehensive workflow how to score and report animals with a harmful phenotype to the authorities. After one year, we reviewed the system from different users' point of view (researcher, animal caretaker, animal welfare officer, designated vet) and could conclude that the introduction of LAVAN overall fulfilled all initial goals.

PE12

The 3Rs database programme: Humane endpoints website and interspecies database

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Abstract

The availability of databases with specific 3R information may save time within the process of compliance to the 3Rs implementation, as required according to Directive 2010/63/EU. To be successful, these databases should be easily found, accessed, managed and updated. Designing, building and filling a database is a time and money consuming activity. Often, financial support can only be obtained in the development stage, whereas continuous updating and maintenance is essential for the sustainability of the databases. Continuous financial support is therefore crucial.

The 3Rs-Centre Utrecht Life Sciences (ULS) has initiated the 3Rs Database Programme, which aims to provide up-to-date 3Rs information free of charge, thereby contributing to the implementation of the 3Rs in research. Among others, the programme currently offers the Interspecies Database and the Humane Endpoints website.

The **Interspecies Database:** (www.interspeciesinfo.com) provides insight into physiological, anatomical and biochemical parameters of different animal species and humans. With the database, researchers can design their experiments smarter with respect to the choice of an animal model. This could lead to a reduction in the number of experimental animals.

The **Humane Endpoints website:** (www.humane-endpoints.info) provides information and training modules on how to recognize and apply humane endpoints in laboratory animals. This helps to prevent unnecessary pain and distress in the animals. Therefore, the website contributes to refinement.

To guarantee a sustainable future for these websites and increase their usage, the 3Rs Database Programme is inviting partners who are willing to cooperate and support its activities.

More information: www.uu.nl/en/3Rsdatabases or 3RsCentreULS@uu.nl.

PE13

Compliance monitoring in a laboratory animal facility using an in-house rodent tracking database system

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Abstract
Animal research establishments are required to continuously monitor and document assurance of compliance with animal welfare policy and legislative standards on animal care and use. In Australia, researchers must be familiar with the Australian Code for the Care and Use of Animals for Scientific Purposes 8th Edition (2013) which promotes the ethical, humane and responsible care and use of animals for scientific purposes. Animal monitoring, including recording reproductive performance of breeding animals, is undertaken to assess, or to ensure the assessment of the wellbeing of animals in accordance with the Code. Monitoring occurs at various levels to include shared responsibilities between the investigators, the animal technicians, the Animal Ethics Committee, the Veterinary Officer and the regulatory bodies. This presentation describes how compliance is achieved in a medium-scale laboratory animal facility by using a reliable rodent tracking ''Rodtrak'' database system which was developed in-house. It shows the monitoring processes during post-approval and when dealing with sick animals and surgical cases including litter monitoring for welfare assessment of genetically modified mice. The "Rodtrak" database is being maintained with the idea of a paperless technology and mainly to have an efficient and user-friendly computerised system of tracking, monitoring and reporting animals listed in approved animal ethics projects. Input from all users of the animal house database is important especially during the design and development phase to ensure that medical records capture required information that meets performance standards of adequate veterinary care.

PE14

ELLI-health notification system for enhancing animal welfare

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Abstract
Simple and efficient tools are needed to activate researchers to react and resolve animals' health issues. We at UTUCAL use ELLI record-keeping system created exclusively for managing laboratory animal facilities. ELLI includes a real-time notification system for recording and alerting clinical observations with a picture or video.

The system alerts using a browser-based interface according to a three-tier scale (+urgent, ++very urgent, +++extremely urgent) by e-mail and/or SMS. Depending on the urgency, researchers must react to status of the individual animal within 120, 72 or 24 hours, respectively. We analyzed the health notifications and reaction times from 2016, 2017 and 2018. The number of rodents in our facilities was 60520, 56339, and 46619 of which the percentage of animal health notifications were 1.35%, 1.32% and 1.59%, respectively. Of those, the percentage of +urgent, ++very urgent, and +++extremely urgent categories were 55-70%, 23-36% and 7-9%. Notifications for coat and skin conditions (wounds, bites, fur) ranged between 34-44%; appearance and body conditions 38-42%; behavior and moving abnormalities/tremors 9-12%; eye abnormalities 7-8%. The average reaction times to different notification categories remained within the maximum allowed: +26-36h; ++23-43h; +++5-9h, respectively. ELLI's health notification system has become a significant tool for facilitating communication between researchers and animal caretakers thus improving animal welfare and reducing suffering. The veterinarian and the animal welfare body get valuable information that helps addressing certain health conditions and implementing the 3Rs.

PE15

Data collection using RFID technology: Improving animal welfare and study documentation efficiency and accuracy

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Abstract
Accurate and timely data collection is critical when conducting any research project. This seminar or poster will focus on the importance of accurate data collection in a new innovate way. We will show how complete passive electronic data collection from the time an animal is weaned through necropsy and tissue analysis creating an individual animal record as part of the larger study documentation increases efficiency and accuracy reducing the number of errors and missing information. We will introduce advanced RFID microchips and custom software for tracking all elements of a research study from simple applications such as high throughput body weight screening, minor surgical modifications, major surgical disease models, medical device implants, PK/PD research, and short and long duration toxicology studies. The goal of this seminar is to demonstrate best practices for efficient and accurate data collection in many different laboratory settings and show the impact of good data collection and the cost of poor data collection. We will focus on a particular area of research, surgery, to demonstrate how this new method for data collection has improved in reducing, refining and replacing animals and how it can aid training technicians. The Participants will take away a better understanding of new innovative data collection techniques and the importance of efficient methods to complete this often monumental task. The target audience for this seminar will be research technicians, study directors and investigators.

PE16

Observing, reporting, treating clinical issues in our animal facility: Improving the culture of care

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Abstract
In order to improve the culture of care at our animal facility (hosting about 11'000 mice, 250 rats and 20 rabbits), a new procedure was implemented to systematically observe, report and treat clinical issues[1].

- Daily visual and weekly cage-changes checks by animal caretakers (AC) enable the prompt identification of new clinical issues and their easy follow-up. For compliance with Swiss regulation and enhanced visibility the affected cage is tagged with a red card.
- New clinical issues are reported daily by senior AC to principal investigator (PI) and attending veterinarian (AV) by e-mail. After clinical examination, AV informs PI about differential diagnosis and discusses potential treatments and more specific tests. In case the animal is moribund, a decision is taken immediately by AV.
- All clinical cases are registered in an internal database reporting: date of observation, building, room, rack position, cage number, animal ID, strain, background, gender, date of birth, PI, symptoms, actions.

From 01.02.2018 to 31.12.2018 we have recorded 1'971 clinical cases; most prevalent are death 29%, wounds 8%, runts 7%, dental problems 6%, eye problems 6%, rectum prolapse 4%, dermatitis 3% and hydrocephalus 3%. The introduction of this new process brought clear improvements in terms of: animal welfare, health monitoring, severity classification for genetically modified animals, and continuous education of AC.

Thanks to the joint effort of AC, veterinarians and scientists, this new procedure built up a culture in our Institution, which improves quality of animal care, compliance, awareness, engagement and finally enhances internal validity of experiments

PE17

From mouse to man

Cubitt Steven
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Abstract
Gene therapies, stem cell therapies and personalised medicine are starting to transform treatments for a number of different debilitating diseases/conditions. What has also been transformed is the speed that these therapies are being transferred from mouse to man. These developments require new types of GMP facilities which feed back into pre-clinical research needs. This presentation will give examples of these projects and discuss the direction of travel for biomedical research.

PE18

Digitalization and automation in the animal house by means of the internet of things

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Abstract
The emphasis of the procedure lies on the execution of physical processes and the nearly simultaneous documentation at the location where data originate. Objects in animal rooms with electronically readable tags attached can reveal themselves with all required needs for studies. In the context of the Internet of Things a procedural software would turn out to be much too complex to be handled and to be learned efficiently by its users. The alternative paradigm is so-called declarative software where facts can be loaded into the memory of an expert system and trigger actions represented by transactions, messages, warnings, help texts, SOPs and even robotic actions. A graph hierarchy of disciplines which can occupy locations as a logistical structure comprising the scientific objects of interest filtered by the licenses, plays an important role in evaluating the facts including user access and manipulation rights. There is a massive advantage of graph databases compared to relational databases due to the avoided ''table hopping''. The engagement with such intelligent systems opens up new opportunities with very practical output, such as documentary transactions inside isolators and/or completely new clean room concepts integrating mechanics and robotic concepts. Researchers can document new crosslinks between scientifically nodes still staying holistic in the system. Rules of the expert system can be institutionalized to the extent that an institution wishes to manifest its know-how and expertise on its data. This can speed up our research significantly and make us a competitive player in a more and more aggressive research environment.

PE20

3R Consortium in Finland

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Abstract
The National Committee for the Protection of Animals Used for Scientific and Educational Purposes in Finland was established shortly after the Directive 2010/63/EU came into effect. As one of the first tasks, the committee organized an enquiry distributed to people working with laboratory animals, cell culture and *in silico*

methods, to find out their level of knowledge of the 3Rs. Based on the results, a 3R working group was established within the committee, to further analyze the need for continuous education and possibilities to share the 3R information.

As the first activity, the group organized four courses, the topics of which were pointed out in the enquiry: *In vitro* models, Ethical principles in using laboratory animals, *In silico* -training, and Genetic disease models and species selection. Researchers using and developing *in vitro* and other replacement models, and the responsible persons from universities, pharmacy and other stakeholders using animals, have been surveyed and called together, to make further plans of a 3R Consortium in Finland.

The Consortium aims to act as a national focal point on the 3Rs, promoting the 3Rs on national level. It will organize education, share information and promote co-research projects. Furthermore, a 3R Award will be announced, to encourage people actively develop new methods and means of research.

The 3R Consortium will consist of institutions such as universities and industry performing animal experiments and developing non-animal methods and approaches. The coordinator of the consortium is FICAM, The Finnish Centre for Alternative Methods, Tampere University.

PE21

Charité 3R – The 3R Center of Charité Universitätsmedizin Berlin

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Abstract
Established in 2018, Charité 3^R aims to bundle and coordinate the 3R research activities at Charité Universitätsmedizin Berlin for a better translation in biomedicine (1).

The center is committed to finding the best therapies by using animal-free methods whenever possible, establishing meaningful human disease models, and increasing animal welfare. Adopting a department overarching organisational structure, Charité 3^R supports researchers with their 3R projects, implements 3R in education and communicates the challenges, needs and opportunities of 3R research to the public.

Charité 3^R is part of ''Berlin 3R'', an upcoming network that is formed together with the Berlin-Brandenburg research platform BB3R and further partners in Berlin, aiming at consequently enhancing the 3R activities within the powerful biomedical research institutions of the German capital.

PE22

Centre for Biomedical Resources large animal facility

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Abstract
The Centre for Biomedical Resources, CBR, at Linköping University, Sweden has built a new and modern large animal facility with the 3R's in focus and to promote animal welfare as well as improve the work environent for both researchers and our employees.

The facility includes three large animal rooms with adaptive pens that can hold calves, pigs, rabbits and guinea pigs. These rooms are connected to a large outdoor pen so that larger animals may choose to go outside for excercise and to perform natural behaviours. The animal rooms are also equipped with an automated water supply and waste disposal system facilitating daily cleaning.

The facility has two state of the art surgical theatres with multiple anesthesia and monitoring stations, enabling simultaneous anesthesia of up to eight animals. There is also a high end visualization center (CMIV) where various techniques such as MRI, Force Dual Energy CT and, as one of the first in the world, Photon Counting CT are accesible for large animals.

We have a close collaboration with the Swedish Defense as well as various medical professions and help them with training of their skills. We are well equipped to hold various types of education and training events with multiple meeting and lecture rooms. Here in this poster we present pictures and information about the facility to encourage collaborations.

PE23

Vital Tissue, an initiative to supply viable human materials to laboratories in The Netherlands

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Abstract
Introduction: Research aimed at human medicine and safety testing is frequently done in laboratory animals, with limitations regarding extrapolation to humans. For the development of better translational methods, viable human materials are often preferred over animals and immortalized cell lines. Especially since several human (tissue specific) models have become available by recent scientific developments (e.g., based on human tissue or iPSC derived cells/organoids). However, limited access to high-quality viable human tissues is a hurdle for many researchers.

Preliminary steps

The initiators of the project have interviewed stakeholders and conducted online polls to conclude that there is a substantial unmet need for various types of human tissues and that potential donors would generally consent to the use of left-over tissues after medical procedures. Next, the initiative was widely supported and extended to include multidisciplinary expertise and multiple organizations.

The Vital Tissue feasibility project

The project aims to prepare a platform that will supply donated tissues to end user laboratories in a (financial) sustainable and transparent way. For this, it will address the legal, ethical and logistic restrictions that may hamper the supply chain. The framework for the long-term qualification of suppliers and end users will be developed to warrant regulatory compliance and biosafety. The needs of end users will drive the system with implications for sample specification (tissue and donor inclusion criteria), logistics, tissue quality preservation, and information management. Project governance includes the involvement of societal stakeholders and high level public information on the development of the tissue supply chain.

PE24

The current status of laboratory animal law, regulation and guideline in Taiwan

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Abstract
Taiwan drew up the Animal Protection Act on 1996 and, experienced 12 times amendments until now. There are 4 articles deal with laboratory animals within 24 articles. ''Guideline for the Care and Use of Laboratory Animals'' was issued by Council of Agriculture (COA) on June 2018 and all institutions with scientific application of animals need to observe it. COA demands all facilities to submit the annual report before end of March every year. The governmental oversight on the institutions is conducted by experts in laboratory animal science and welfare since 2003. More than 200 institutions participated in animal research in 2018, including universities?/ colleges 32.8%, animal drug companies 8%, pharmaceutical/ biotechnology companies 10%, and hospitals and research institutions 44% as well. One tenth of these facilities had been AAALAC accreditated since 2000 and commit to high well-being for animal care and use. The law, regulation and guideline address the minimum standard requirement for human care and use of laboratory animals, compared with Pacific Rim, Western and European countries, we still need more efforts to improve among awareness of animal welfare, animal protect and ethical concerns to enhance animal well-being by continuous evolvement in Taiwan.

PE25

Reporting quality of animal research in Slovenia

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Abstract
Nontransparent reporting has been recognized as an important factor contributing to reduced value of publications, increased waste of financial resources and unjustified use of animals. To improve reporting quality and increase the reproducibility of animal studies, the ARRIVE guidelines and Gold Standards Publication Checklist (GSPC) were published in 2010. However, in Slovenia those two documents have not been noticed until 2017, when Reproducibility issues in animal research were exposed at the congress of Society for Laboratory Animals of Slovenia (SLAS). SLAS is a young society aiming to increase the awareness and knowledge of scientists on laboratory animal science (LAS) from 2010 on. To evaluate the influence of SLAS activities on scientists we analyzed the reporting quality of representative samples of animal research papers published by various Slovenian research groups before and after SLAS activities, i.e. in a period of 2005-2008 (before SLAS) and 2013-2016 (after SLAS). Since SLAS has organized workshops and congresses addressing fundamental topics of LAS, comparison was based on Figure1 of GSPC, which schematically lists items exposed by SLAS past activities such as legislation, environmental, dietary, microbiological and genetic factors.

Results have shown slight improvement in reporting quality of Slovenian scientists, particularly on items addressed by SLAS in the past, which indicates that SLAS events have importantly influenced the scientists. However, a lot of improvements still should be done in this area in the near future. SLAS events or other forms of continuous education are necessary and indispensable in the furtherance of LAS among researchers in Slovenia.

PE26

Evaluation of research and education projects: Experimental, Educational and Research Center ELPEN (1996-2019)

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Abstract
Background: The Experimental, Educational and Research Center ELPEN was founded in 1996, to serve the national and international biomedical research community. Its size and character have evolved during the 23 years of operation.

Objectives: To record and evaluate research and education projects in our facility.
Methods: We analyzed project authorization and animal use records from 1996 to 2019.
Findings: More than 570 project licenses have been issued during 1996-2019. The majority were for research projects, leading mainly to PhD and Master theses, and the others for health professionals' training projects. Research projects began conservatively in 1996 using rats and launched from 1999 using swine, rabbits, rats and mice; with an average of 20 new projects per year. Since 2011 the Center has granted 146 scholarships. Research findings have resulted in more than 300 PhD theses, 300 scientific publications and 200 awards. Involvement of the Center in medical education began in 2000 while the number of training projects sprang in 2011, following the inauguration of new facilities. More than 900 health professionals' training courses have been held, providing training for more than 25,000 participants. The Center is an accredited member of UEMS/NASCE.
Conclusions: The Experimental, Educational and Research Center ELPEN provides a hub for the progress of biomedical research and advancement of medical training in our country. It is a successful model of interprofessionalism, interdiciplinarity and collaboration of the private and public sector.

PE27

The Danish National Committee for the protection of animals used for scientific purposes

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Abstract
The Danish national committee (DNC) was established in 2013. The DNC members are also board members of the Danish 3R-Center (www.3rcenter.dk). The DNC and the 3R-Center have a joint secretariat with The Danish Animal Experiments Inspectorate. This structure is ideal for knowledge sharing. The Danish national committee (DNC) hosts an annual network meeting for the Animal Welfare Bodies (AWBs). The meeting includes a workshop where AWB representatives exchange experience. The meeting also hosts a popular market place for presenting and sharing 3R-ideas from the everyday work with the animals at the facilities. No 3R-initiative is too small to be shared. Several times participants have taken home an idea from one meeting only to develop it further at their facility and present the improved version at the next meeting. In collaboration with The Animal Experimentation Council the DNC has written guidelines describing standardized procedures, which are often part of applications to carry out experiments on animals. These procedures and their impact on the animal can be expected to be approved within the described limits as part of an animal experiment, unless the application contains other special conditions which would militate against approval, such as, for example, the existence of a less harmful method of achieving the same objective. The members of the DNC and the 3R-board are: Chairman, Christine Nellemann, Erwin L. Roggen, Lisbeth E. Knudsen, Adrian Smith, Jan Lund Ottesen, Axel Kornerup Hansen and Peter Bollen.

PE28

Report of the Greek National Committee: first 2-years activity

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Abstract
Background: The Greek National Committee (NC) for the protection of animals used for scientific purposes was established in September 2016, with seven members including laboratory animal veterinarians, biologists and a bioethics expert. It completed its first 2-year term in September 2018 after ten face-to-face meetings and many e-communications.
Activity: During this period the NC

- communicated with the Greek Animal Welfare Bodies and Protocol Evaluation Committees of breeder, supplier and research establishments, provided information and advice
- communicated with the European Commission (EC) DG Environment, reporting its output and recommendations produced, which were uploaded in the secured EC platform
- created a NC webpage in the Ministry of Rural Development & Food's platform, where it uploaded recommendations regarding the presentation and publication of research studies conducted on small and farm laboratory animals, their transportation, as well as guidance documents
- revised the Project Application, Non-Technical Project Summary (NTPS) and Retrospective Review forms, which were disseminated by the Ministry throughout the country
- organized the collection and publicizing of NTPS on its website
- prepared the draft Ministerial decision on the procedures and requirements for the education & training of persons conducting the four functions, the institutions and the courses' content
- prepared draft education & training guidelines with medical scientific societies that use animals for practical training
- proposed one of its members for the FELASA WG on Farm Animals
- provided its expertise to the recently established NC of Cyprus.

Conclusions The Greek NC was impressively active during its initial incumbency.

PE29

Implementing 2010/63/EU Directive in University of Debrecen Committee of Animal Welfare's work

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Abstract
The EU Directive 2010/63/EU of the European Parliament and of the Council defined the tasks of Animal-Welfare Bodies (Article 26 and 27). In Hungary the Decree 40/2013 (based on EU Directive) of the Hungarian Government established similar tasks and these are supplemented by the following: preparing and monitoring the implementation of the institutional rules on animal experiments; organizing training and education programs in laboratory animal science for personnel (EU ¨B¨ and EU ¨A¨ level, animal caretakers); supervising the authorization of scientific projects (University level); administrative function (registers, animal records). In the last twenty years these tasks at University of Debrecen (UD) were carried out by Committee of Animal Welfare (UDCAW).

Based on the Directive one of the most important assignments of the Committee is to implement the 3R's (Replacement, Reduction, Refinement) in the University of Debrecen's scientific work and education. To fulfil this requirement new core modules, function specific modules and additional (task specific) modules were introduced in the Laboratory Animal Science and Welfare Courses' topics. According to the new regulations in 2016 the Courses were accredited by National Scientific Ethical Committee on Animal Experimentation.

The UDCAW's activities (work, participation of representatives at national and international laboratory animal science conferences) are presented in form of summary reports at meetings of Animal Research Committee of Hungarian Academy of Science regularly. The results of the Committee's activities are also recognized internationally.

PE30

3R program at the Comparative Medicine and Bioimage Center of Catalonia: Driving responsible research

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Abstract
The Comparative Medicine and Bioimage Centre (CMCiB) is an academic new highly technified facility of Germans Trias & Pujol Institute, devoted to multidisciplinary biomedical preclinical research and continuing education of surgeons. A wide range of research projects are carried out, in an environment of bioethics promotion and 3R application policies. There is a 3R programme that includes the following areas: The institution has implemented a drosophila fly room laboratory to promote its use for translational research. 70% of diseases affecting humans have their equivalent in drosophila: diabetes, Parkinson's disease, cancer, infectious diseases. The first result has been the specie replacement of a drug potency test previously done with micel.

The refinement of procedures and the reduction on animal numbers is promoted with the use of bioimaging and diagnostic imaging techniques: Luminescence, fluorescence, micro-CT for small species and 3T MRI, fluoroscopy and ultrasound for haemodynamic; as well as 3D-laparoscopy and endoscopy for minimally invasive surgery in large species.

Surgeons training and medical device design and testing often require the use of animal models. The inclusion of new interactive audio visual technologies and multimedia equipment in the facility allow recording, video conferencing and streaming that promotes the diffusion of the training to more professionals.

Finally the bioinformatics department develop mathematical models maximizing the information that can be obtained from experimental work through the improvement of their interpretation, allowing more conclusions to be obtained. This approach has obtained economical suport of a private company for 5 years. Examples are shown in the poster.

PE31

Implementation of an animal-welfare body in dog and cat experimental facilities

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Abstract
According to the articles 26 & 27 of the EU Directive 2010/63, institutions must set-up animal-welfare body (AWB) to follow the development and outcome of projects, to assist the users in internal operational processes and to advise them on opportunities for the application of the 3Rs within the projects. Oniris' Nutrition, Pathophysiology and Pharmacology Research Unit (NP3) undertakes and leads research with industry partners across France into the health, nutrition, and management of experimental cats and dogs. The Cat and Dog facilities of NP3 Unit has an AWB that is composed of a scientist, a veterinarian, an animal caretaker and an ethologist. In order to follow the development and outcome of projects, and to demonstrate to regulatory authorities that the welfare of animals is maintained to current best practice, the Pet AWB at oniris adopts a team approach using the moodle paleform to facilitate the data entry (*i.e. recording events when they occur during the project*) and to share information (*i.e.* opportunities of training, notification documents. . .) with all those involved, like the technicians and the researchers. Specific management practices that provide for optimal care, handling, housing and nutrition, and some events aimed at improving welfare of Cats and Dogs will be presented and discussed.

PE32

The animal-welfare body advices: Experience feedback from a plateform of experimental surgery

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Abstract
The minimum functions of animal-welfare body (AWB), according to the articles 26 and 27 of the EU directive (2010/63/EU) include advising on the care and use of animals and implementing the three Rs, assisting in internal operational processes, following the development and outcome of projects, and recommending rehoming schemes. The experimental surgery plateform of Oniris (veterinary school of Nantes, France) has the objective to support the user in the experimental surgical procedures as well as to provide accommodation for the maintenance, care and welfare of experimental animals. Rabbits, pigs, small ruminants and horses are commonly used in this platform. To optimise the animal welfare and validity of the individual projects, the users of the platform build on advices provided by the AWB. The team of the AWB includes two veterinarian and scientist and an animal caretaker who possess complementary knowledge (i.e. *anaesthesia and pharmacology*), experience and motivation. The team's advice is particularly relevant to the refinement strategies such the timely and accurate recognition of the pain and the implementation of suitable anaesthetic/analgesic regimens. Some events like rehoming rabbits and others all aimed at improving welfare of rabbits and pigs included in surgical procedures will be presented and discussed.

PE33

Collaborative platform for respiratory route toxicology and exposure to electromagnetic fields

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Abstract
INERIS has developed a collaborative platform dedicated to *in vivo* rodent toxicology, with a focus on method standardization and the development of alternative methods. This state-of-the-art facility of 1000 m^2 is composed of mice and rat housing areas and innovative experimental installations, allowing exposure to chemical agents, including nanoparticles, as well as to physical agents, such as electromagnetic fields.

Equipments and rooms are dedicated to studies using the respiratory route (nose-only or whole-body exposure). This includes a complete rat inhalation system devoted to exposure to nanoparticles, composed of nose-only exposure chambers, associated metrology for full aerosol characterization, and nanoparticle aerosol generators. In addition, these generators and metrology system can also be associated to an air-liquid interface Vitrocell™ system, allowing *in vitro*/*in vivo* comparisons or *in vitro* research of underlying mechanisms. Results from *in vitro*/*in vivo* comparison performed with TiO2 nanoparticles will be presented.

A separate area of the facility is dedicated to exposure to electromagnetic fields and the assessment of their effects on health. This includes anechoic reverberating chambers allowing controlled metrology and continuous exposure to environmental doses of radiofrequency electromagnetic fields. Effects of electromagnetic fields on the central nervous system and on reproduction are investigated. Preliminary work and results will be presented.

Aside of these dedicated equipments, standard general toxicity, pulmonary, reproductive and neurologic toxicity of environmental chemicals, can be evaluated, with available complementary *ex vivo* and *in vitro* analytical (biochemical analyses, histology, etc.) facilities. This facility is GLP-compliant, and therefore also suitable for regulatory studies.

PE34

The recent trend of minipig research in Korea: Designed Animal Resource Center

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Abstract
Designed Animal Resource Center (DARC) is an infrastructure facility to support research activities employing experimental animals at Institute of Green Bio Science Technology, accommodating up to 150 mini-pigs, 300 primates and 3,000 cages of rodents. DARC develops, takes care of and distributes 1) designated pathogen free (DPF) mini-pigs applicable in the national biomedical / regenerative medicine fields through its world-class pig core facility, 2) various rodent models to investigate and prevent human and animal diseases as a national center for experimental rodents, while 3) raising and distributing primates to be used in research, collectively promoting development of new bio medicines, organ research, and support technologies. Furthermore, DARC has an internationally recognized clean animal rearing system which accelerates the development of high-tech life science research, internally and externally playing a role as the leading institute in the field. In addition, DARC leads regenerative medicine through multi-disciplinary cooperation with various study fields including medical science, agriculture, biology, cell engineering, and develops the livestock industry by fostering skilled manpower for generation of future fusion technology and high-value animals.

PE35

Biobank at Biomedical Primate Research Centre, The Netherlands: Attractive alternative source for scientific research

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Abstract

Tissues and genetic material obtained from non-human primates represent a valuable resource in biomedical research. Offering and obtaining this type of material is limited, time demanding and needs high expertise. As pressure grows for reduction of numbers of animals used in research, the Biobank is becoming an attractive alternative source for testing scientific ideas, new vaccines and biologicals before proceeding to pre-clinical studies.

BPRC's Primate Biobank is the biggest nonhuman primate Biobank in Europe and it is based on the principals of the 3Rs: refinement, reduction and replacement. The aim of the Biobank is to provide rare and valuable primate specimens for internal use and for external scientists who can use the material for biomedical research as well as for conservation studies. The primary focus is on the following primate species: Rhesus macaques (*Macaca mulatta*), Long-tail macaque (*Macaca fascicularis*) and Common marmoset (*Callithrix jacchus*). Samples from endangered species such as great apes – Common chimpanzee (*Pan troglodytes*) and Orangutan (*Pongo Borneo*) are also collected.

The Biobank consists of:

1. Tissue bank: organs, tissues and cell lines. Organs without morphological alterations from clinically healthy retired animals and tissue samples from animals of different species with experimentally induced or spontaneously developed pathological conditions and syndromes are available. The tissue collections include frozen samples stored at -80 C, samples fixed in formalin, paraffin embedded tissue blocks and cell lines frozen and stored in liquid nitrogen.
2. Serum and Gene bank: Serum, DNA, RNA, cDNA samples are adequately stored at −20 or −80°C.

PE36

Keep an online platform for laboratory animal science running – LAS interactive novelties

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Abstract

The learning platform for laboratory animal science *LAS interactive* exists since more than 10 years. Its maxim is to enable the LAS community to share their collective knowledge and expertise in husbandry and working with laboratory animals in respect to the 3Rs. In this regard the platform has been largely funded by public grants during the last years (DFG). Presently, *LAS interactive* provides information on 16 species and various experimental techniques.

To keep the concept of the e-learning platform alive, a close cooperation with LAS experts is a prerequisite. The cooperation partners impart actuality and enlargement of the learning content. This way, even information about less frequently used animals in research like dog and sheep has been compiled recently.

Authors can decide whether their content will be used for their own teaching purposes only, or if it will be visible to all registered users. The arranged learning packages are convenient to qualify personal for working with animals. To satisfy national as well as European recommendations, the learning packages are adapted to country-specific requirements. Complex text passages have been equipped with quizzes to promote the reflection of sophisticated topics (ethics, biometry). Videos and slide shows illustrate best practice and interactive games convey content in a playful way. Flashcards are intended to support students aside from the e-learning platform itself.

Hence, LAS interactive can pursue its collaborative approach and will continue the contribution to sharing knowledge and expertise within laboratory animal science in a high quality and up to date manner.

PE37

Close at hand: Animal welfare officers are important mediators of the 3R concept

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Abstract

The complex duties of animal welfare officers involve a high degree of responsibility. Determined by the tasks assigned to them, they are caught between maintaining the best possible

animal welfare on the one hand and ensuring scientific progress on the other. They act as intermediaries between scientists and authorities. In this process they have to take a neutral attitude, but in doubt have to decide in favour of the animal (*in dubio pro animale*). Their advices should be based on the 3R concept and should be oriented in such a way that, in terms of replacement, alternative methods should ideally be used, in terms of reduction as few animals as possible should be calculated and, in terms of refinement, the gentlest method should be applied. Thereby, animal welfare officers exchange information very closely with scientific experts and authorities, which requires an overall expertise in these areas. The majority of animal welfare officers are trained in veterinary medicine and therefore have a good overview in the area of refinement. However, there is still a strong need for action with regard to advice on the use of alternative methods. In order to fully meet the requirements of Russell and Burch, the 3R Centre Giessen (ICAR3R) develops concepts in order to close this gap. If this is successful in the foreseeable future, the role of the animal welfare officer will actually be strengthened, as they can make a major contribution to the sustainable implementation of the 3R concept in basic biomedical research.

PE38

Laboratory animal professionals and the ethical review of clinical studies with client-owned animals

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Abstract
Clinical studies with client-owned animals are important for veterinary medicine and animal health in general but also as translational studies in finding correlations with human health issues. This expanded cross-species approach potentially alleviates the problem when new drugs successfully discovered in laboratory animals fail in human trials. With an increased understanding and awareness of ''One Health'', the public in general and scientists specifically recognize the value of comparative medicine. However, in contrast with laboratory animal research where regulatory oversight is intense and ethical review of laboratory animal use is a requirement, enlisting client-owned animals in clinical studies is not clearly regulated. Bioethical review of laboratory animal use protocols or study plans is common now in many countries and the expansion of this practice at a world-wide level e.g. through AAALAC International accreditation, is a welcome and needed development. Several publications in the past 5 or 6 years have pointed to the need to apply principles of ethical review to clinical studies as well. Personnel involved in the ethical review of laboratory animal use have contributed to this evaluation of clinical studies and their continued and increased involvement represents a great asset in this field. These individuals have a lot to offer and their contributions should be enlisted. A brief overview of the present (legislative) situation and the above points will be presented and reviewed with the hope to engender needed continued discussions with regulatory agencies, professional organizations and the public, including members of the laboratory animal science and medicine field.

PE39

The RepRefRed Society

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Abstract
Within the last years, animal welfare and animal ethics have risen to essential topics in the field of biomedical research. Since animal well-being is assumed to have major impact on research results, together with the revision of the law for animal experiments in Austria 2012 the 3Rs (replacement, reduction and refinement of animal experiments) became a crucial theme for any person involved in animal studies. To critically analyze animal experiments and husbandry, as well as to encourage alternative means, we founded the association "Verein zur Förderung von alternativen Biomodellen" (RepRefRed society) in 2016. Together with veterinarians, animal-anaesthesiologists, animal keepers and scientists the RepRefRed society organizes congresses, meetings as well as workshops and offers a platform to facilitate knowledge transfer in the field of alternative methods. Thus, the RepRefRed society contributes to enhance animal study protocols, allows scientists to learn innovative alternative techniques and promotes replacement, reduction and refinement of animal experiments.

PE40

Transparency agreement on animal research in Portugal – An overview

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Abstract
The Transparency Agreement on Animal Research in Portugal is an initiative proposed by European Animal Research Association (EARA) in close collaboration with the scientific community to promote accurate and up-to-date information about the use of animals for research in life sciences. This project was based on the previous examples in Europe, the Concordat on Openness on Animal Research in United Kingdom (2014); the Transparency Agreement on Animal Research in Spain (2016) and the Statement in Support of Animal Research and a Transparent Approach in Belgium (2016). In Portugal, 16 organizations including Research Centres and Universities, signed this agreement in 2018. The signatories are committed to promote access to a

comprehensive body of information, explaining what animal research involves and the role it plays in scientific findings, new therapeutics development and regulatory testing. To achieve that, institutions are expected to fulfil four commitments. The First Commitment is to focus on the need to speak clearly about when, how and why animal models are used in research; the Second Commitment is to enhance communication with the media and the public; the Third Commitment focuses on the need to be proactive in providing opportunities for the public to find out more about animal research; the Fourth Commitment is on the need to report publicly on progress and share experiences. EARA is working together with Portuguese institutions to foster a climate of openness and to make sure that society can discuss issues related to animal research from a position of knowledge of the facts.

PE41

Animal studies at the University of Leuven: Let's talk

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Abstract
As a leading university in Europe, KU Leuven engages in innovative biological and biomedical research and education, which, if necessary, involves laboratory animals.

Although animal studies are carefully planned and subjected to critical ethical evaluation and animals are treated with compassion and respect, animal testing still receives a lot of criticism. Often ignorance and misinformation about the research goals and the use and fate of the animals lies at the basis of this incomprehension. Therefore, KU Leuven and its Laboratory Animal Center decided to openly communicate with the interested public about animal research.

Recently an important step was made by publishing a new public website "Scientific research with laboratory animals at the KU Leuven" where firsthand information regarding the research projects with animals, the 3R's, regulations, ethics, animal welfare... is provided. In addition, a report was published on the activities in 2017 giving insight in the number of animals used in studies, the number of evaluated projects and highlighting some scientific achievements. The Laboratory Animal Center also organizes guided tours for staff members, KU Leuven personnel and their family, and for secondary school students.

Transparency, openness, communication and dialogue on animal research with the public is challenging. We hope that, by explaining how and why animal research is carried out, we can reduce misconceptions and myths, and improve understanding and acceptance. The feedback we receive is of value to improve our institutional regulations and perhaps even more importantly for our training programs in order to improve our culture of care.

PE42

LIVE 3R – Transparency starts now!

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Abstract
Coping with animal experimentation, the demand for open communication practice is increasingly rising. This is due, to the fact that scientists have the need to share their knowledge with the community regarding experiences with procedures that have had negative or positive effects on the welfare of laboratory animals. However, so far there are limited options for communicating this. The reporting of such events often do not fulfill the requirements of scientific journals, making it difficult to disseminate this knowledge. For this reason, the internet platform LIVE3R was launched.

LIVE3R intends to optimize exchange and thus supports the ethical assessment of animal experiments. Thereby, the platform offers three access-points: REPORT provides the possibility of describing unforeseen negative events during animal experiments. REFINE maps all aspects of refinement procedures aiming on stress minimization and enhancement of animal welfare. RESCORE intends to collect score sheets which were tested to be optimally adapted to specific animal models.

Contributions will be proven to be scientifically valid, and they will be accredited with a training period of two hours. The benefit of LIVE3R is based on active implementation of the 3R concept. In the sense of Russell & Burch, involved persons get the opportunity to provide input to the 3R principle and to share knowledge with others. Negative repetitions can be avoided, and beneficial refinement methods can find broader application. Overall this strongly enhances to the sustainable long-term improvement of animal welfare.

PE43

Use of a live virtual tour to increase openness in a pharmaceutical animal facility

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Abstract
We are a signatory to the Concordant on Openness, an initiative from Understanding Animal Research (UAR) that over 120 UK facilities have joined. Each organisation must demonstrate its commitment to transparency regarding the inclusion of animals in our research. Having signed up, we need to produce evidence of

our commitment to being open about animal research, which includes raising awareness of the inclusion of animals to the public and our work colleagues and exchanging ideas and work practices across the industry. In order to do this, we highlight areas where we already demonstrate openness, for example: monthly facility tours which are open to everyone who works in the company; including animal statistics in our responsible business supplement report and having a page on our public facing website which focuses on animals in research; work experience students coming to work in the animal facility for a week. Recently we have focused on a new initiative called the 'Live Virtual Tour', which enables us to show a larger audience of people round our facility in real time, opening our doors to a much wider internal audience from multiple sites. This presentation will give an overview of transparency and openness and will include a demonstration of our Live Virtual Tour, exploring the facilities we have, how we take care of the animals, the science we do and increase our transparency and openness.

PE44

True transparency: How to be open and honest about animal experiments

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Abstract
Transparency is of growing importance in working with laboratory animals. Animal welfare NGOs and the public want to know for what purposes animals in experiments are used, how the animals are treated, and how researchers are working on animal-free research methods. Many research institutes see it as both their moral obligation and a way of reputation management to be frank about animal experiments.

Therefore, Utrecht University and the University Medical Centre Utrecht are putting growing effort into communications with the public, coordinated by their joint Animal Welfare Body Utrecht and the 3Rs-Centre Utrecht Life Sciences. In addition to a history of speaking to journalists and camera crews, inviting interested groups of people and NGOs, and publishing annual reports on animal experiments, an integrated strategic transparency project has been initiated, together with partners in Utrecht Life Sciences. The bottom line of this strategy is to be true and transparent. If you can't explain it, it may be ethically wrong.

We would like to present our strategy and exchange ideas and best practices of public communications with our colleagues. Elements of our strategy are:

- online disclosure of all licensed research projects with animal experiments
- sharing animated instruction video's and policy documents publicly online
- a game with stuffed animals to make children and grownups reflect on ethical dilemmas of animal experiments
- a new format of our Annual Report on Animal Experiments for reaching a larger public
- a search for cutting-edge tools to increase transparency

PE45

Innovative nasal filters allow measurement of exposure to Laboratory Animal Allergens

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Abstract
Laboratory animal allergy (LAA) can develop as a result of prolonged exposure to animal allergens. Approximately 20% of animal technicians develop allergy and 10% – serious asthma. UK guidelines state that employers must prevent exposure to animal allergens and monitor the effectiveness of control procedures. The most popular monitoring method is IOM filters attached to a pump carried by the technician, which can be cumbersome. Previous data has demonstrated that various allergens (e.g. cat, pollen) could be captured on a novel type of nasal filter. In this study we investigate the feasibility of using the nasal filters for the assessment of exposure to mouse allergen in an animal laboratory.

Animal technicians wore the nasal filter and the IOM filter simultaneously during normal routine work. Allergen was extracted from the filters in PBS-Tween and levels of Mus m 1 were quantified using a highly sensitive (LLOD=0.01ng/ml) multiplex assay.

Significant levels of Mus m 1 were detected on the nasal filters and they correlated with the type of activity performed and the type of mouse housing. Levels were compared to the suggested 'safe' limit of allergen exposure of 5ng/m^3. The technicians described the nasal filters as comfortable and more practical for routine monitoring than the IOM filter and pump.

These data indicate that nasal filters may be a simple and easily wearable method for monitoring laboratory animal allergen exposure. Future studies are planned to assess the use of the nasal filter for analysing exposure to allergens from rat and guinea pig.

PE46

Animal and zootechnician welfare, have a look on your perception of pain and discomfort

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Abstract
How to establish step by step a program of reconstructive surgery necessary and sufficient to accept the appearance of a disfigured monkey. Following a conflict between tow Macaca mulatta, a 17 years old female, born in captivity (France), is recovered with deep avulsive wounds of the lip and cheek. After a conservative treatment, the staff was suffering from the aesthetic appearance of the animal, which had also resumed perfectly normal behavior and showed no short-term functional deficit. How and why choose a

surgical reconstruction on animals that does not express pain or suffering?

PE47

Where to go with laboratory animals after the trial?

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Abstract
The fate of laboratory animals should be considered when applying for a breeding license or animal experiment already. In many cases, the humane killing of animals is unavoidable, because the survival would be associated with more than minor pain, suffering or damage, the keeping outside genetic engineering facilities is not allowed or organs have to be removed for further examinations. The delivery of carcasses as feed animals for birds of prey or predators can be a reasonable justification for humane killing of surplus animals. Those animals, which restored to health after the trial, may continue living after subsequent veterinary examination. A responsible decision has to be made as to whether re-use in experiments or alternative accommodation seems possible and justifiable. When animals shall be re-used, it must be ensured that none of the experimental procedures that they went through is categorized as associated with severe pain or suffering. If the adoption of small and domestic animals is planned, they must not pose any danger to other animals or humans.

The facility should provide programs for the rehoming of animals, including careful selection of animals and future owners, as well as the preparation of animals for their future living and ensuring adequate housing, nutrition and veterinary care. Cooperation with institutions experienced in the rehoming and adoption of animals may be advantageous. In addition, when delivering animals that might be used in the food chain, the safety of products derived from them must be proven concerning other animals, humans, and the environment.

PE48 WITHDRAWN

PE49

Innovative staffing strategies: A case study on insourcing

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Abstract
For any organisation the most valuable resource are the people that work there. This especially rings true for a Laboratory Animal facility, as having the right people in place with the appropriate skills and qualifications is critical for such a busy and complex environment. Finding, selecting, hiring and making sure people are happy and engaged is a full-time job on its own. So imagine how much more productive a facility manager could be if the staffing aspect was partially or completely taken care of? They could focus more on other important aspects such as working with their research teams, regulatory issues, taking care of equipment and consumables, and ensuring the health status and well-being of the animal models.

Insourcing provides that opportunity. A well-planned insourcing model offers a seamless integration into a vivarium staffing model and provides the quality of service that allows for increased productivity. The presenter will provide an overview of the insourcing staffing model and review case studies that reflect how other Laboratory Animal Science programs have adopted and benefited from this model.

PE50

Developing a global corporate resiliency building program for personnel working with laboratory animals

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Abstract
Compassion stress – the stress experienced when caring for animals that may deteriorate in condition or require euthanasia over the course of a research project – commonly occurs amongst those working with research animals. Unless it is addressed, compassion stress can escalate to compassion fatigue, leading to profound physical and mental exhaustion, and a loss of pleasure in work and daily life. Compassion fatigue can also adversely impact animal welfare as people look to remove themselves and their feelings for animals in their care. To investigate the root causes of the issue further and to develop an impactful corporate program that would meet the needs of personnel across multiple sites, a pre- and post-workshop survey tool was developed and administered to >700 employees attending an internal multi-day compassion fatigue workshop. Results of this survey were used to develop internal compassion stress training and build an internal network of 'resiliency building ambassadors' to extend the program to multiple sites across the organization, focusing on tools for building resiliency, personal wellness, providing tributes to research animals worked with, enhancing communications during research projects, promoting animal adoption and rehoming programs, and sensitizing senior management to compassion stress and their role in helping personnel deal with difficult situations at work. The program includes flexibility for sites to select content to ensure that culturally appropriate tools and materials are available across sites.

PF1

An animal welfare assessment system to monitor the lifetime experience of experimental animals

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Abstract
In keeping with the European Directive 2010/63/EU and UK Home Office requirements for prospective and retrospective assessment of experimental animal welfare, Public Health England (PHE) and Surrey University Veterinary School have collaborated to develop a software system that enables quantitative assessment of the lifetime experience of animals used in scientific experiments. This system, the Animal Welfare Assessment Grid, (AWAG) has been tested using both retrospective and in-life data and has been found to give a clear indication of an animal's welfare during its lifetime. The system scores four parameters that impact on well-being, namely physical condition, psychological state, environment and experimental/clinical procedures. Within each parameter, a number of factors are evaluated to give a numerical score which is then added to give a total welfare score for that particular time-point. The software can provide a visual and numerical summary for animals that can be interrogated to provide evidence of improving or deteriorating welfare. Such data is invaluable in presenting positive changes to housing or experimental design to regulators, ethical review bodies and to staff in experimental facilities.

PHE have obtained NC3Rs funding (https://www.nc3rs.org.uk/development-and-evaluation-animal-welfare-assessment-grid-awag) to refine and trial the AWAG software such that it is easy to install and use and so that it can be applied to individuals or groups in a wide range of species. Here we will present on progress so far and offer the opportunity to familiarise with and trial the AWAG system with a view to applying the system and providing helpful feedback that will assist design.

PF2

An integrated approach of skin lesions estimation after UV irradiation in animal model

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Abstract
In spite of development of in vitro methods as alternative to in vivo tests, the estimation of cutaneous drugs in animal remains mandatory. For the study of photoinduced skin lesions we propose a integrated approach in laboratory animals. We propose to study the general skin state and genotoxic effect after irradiation using a micronucleus test in one experiment on the same animals. This approach reduces the number of animals used in the experiment.

The experiment was performed in female Wistar white rats two months aged. The animals back was shaved (4x4 cm) 24 hours before irradiation. The source of UV radiation was 4 USHIOUV-B 8W lamps (313 nm energy maximum). UV lamps were located at a distance of 15 cm from the back. Two groups of animals were irradiated within 15 and 30 minutes respectively, the control group was under similar conditions apart UV irradiation.

Animals were removed from the experiment on the 4th day after irradiation with deep ethereal anesthesia. The degree of erythema and the thickness of the skin fold were investigated. Then skin samples were taken for further research by micronucleus test.

In our research skin erythema and the fold thickness increase were observed after 15 and 30 minutes of UV irradiation. The increase in the number of cells with micronuclei, in comparison with the control was shown in UV-B-irradiated groups (the percentage (P25%-P75%) of cells with micronuclei was 0,19% (0,18-0,20) for 15 minutes irradiated groups and 0,49% (0,40-0,60) for 30 minutes irradiated groups.

PF3

Assessment of prospective and actual severity of 2014-2018 projects of our establishment

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Abstract
Background: A severity assessment framework, requiring prospective assessment and actual severity reporting for animal research projects, has been implemented in Greece since 2014, following the issue of Presidential Decree 56/2013 (conforming to Directive 63/2010/EU).
Objectives: To identify and assess prospective and actual severity levels in our establishment.
Methods: Data regarding animal species, animal numbers, project purpose, prospective severity and actual severity classification from projects authorized and implemented during 2014-2018 were recorded and analyzed.
Findings: Project purpose included higher education and training (53/101 (52.5%), 100% non-recovery) or basic and translational research on human diseases (48/101 (47.5%)). Research projects were assigned prospectively: 14/48 (29.2%) non-recovery, 24/48 (50%) moderate, 10/48 (20.8%) severe; with 26/48 (54.17%) involving at least one animal assigned different actual severity. However, 78.2% of the animals of research projects confirmed the predicted severity. Actual severity was significantly lower than predicted severity (unit: animal, $p<0.0001$, Wilcoxon matched-pairs signed-rank test). Significant variability in the discrepancies (prospective minus actual severity) was noted between the examined species (unit: animal, $p=0.0001$, Kruskal-Wallis test, species: mice, rats, rabbits, swine). Reasons of discrepancy included: experimental groups or arms with lower severity, no recovery under/after anesthesia, refinements after retrospective evaluation, better response than worst prediction, severe complications or mistaken estimation.
Conclusions: The results confirmed adherence of the project evaluation committee to the guideline of prospective severity assignment based upon the worst prediction. Although processes of severity classification and early retrospective evaluation contributed to refinement, more initiatives to promote reflection, education and publication of information are necessary.

PF4

Animal welfare assessment in an experimental assay of mammary carcinogenesis

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Abstract
This work intended to establish the most adequate humane endpoints in an experimental assay of mammary carcinogenesis chemically-induced in female rats.

Procedures followed the European Directive 2010/63/EU and National Decree-Law 113/2013, and were approved by Portuguese Committee (Approval n.008961). Twelve female Sprague-Dawley rats (Harlan Interfauna, Spain) were divided into two groups: *N*-methyl-*N*-nitrosourea (MNU) (n=10) and control (n=2). At seven weeks of age, animals from group MNU received an intraperitoneal injection of the carcinogen MNU at a dose of 50mg/Kg. The following humane endpoints were proposed prior the beginning of the experiment: body condition; body weight; posture; coat and grooming; mucosal; eyes, ears and whiskers; mental status; response to external stimuli; hydration status; respiratory and heart rate; body temperature; macroscopic evaluation and mammary tumors' burden, tumors dimension). Animals were monitored twice a day and were humanely sacrificed at 25 weeks of age. Data was registered in a table and scored between 0 and 3, and analyzed with descriptive statistics.

No tumors were observed in control group. Six animals from group MNU developed mammary tumors. From these, five animals developed at least one mammary tumor >35mm. No changes were observed in the remaining parameters. All animal welfare scores were maintained below the pre-established limit. No animals were sacrificed before the end of the experiment.

Conclusion pointed that the alteration of a solely endpoint does not imply the animal sacrifice. Clinical evaluation sheets contribute for data collection directly applied to oncology studies and simultaneously may improve overall animal welfare evaluation.

PF5

Use of the Mouse Grimace Scale to assess effects of compounds in preliminary toxicology

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Abstract
The Mouse Grimace Scale was developed to assess pain in mice[1,2,3] and consists of five facial elements that are scored individually and added-up for a final score. It has been shown to be highly accurate, repeatable, reliable and easy to learn. Our objective was to apply the MGS to our experimental work to assess its validity and practicality to be used as a routine animal welfare assessment tool. We started testing its application in a preliminary toxicology protocol that supports efficacy studies by identifying potential adverse events and drug levels in blood. It was selected because it is a routine test in which animals often show a range of mild to moderate sign for several days. Female BALB/c mice were dosed twice daily for four days. They were videotaped at different

time points and MGS was scored blindly from the videos. Appearance and activity (AA) were also scored as validation assessment. MGS score was higher post-dose than pre-dose and it stayed higher for mice showing adverse effects, whereas it went down for vehicle-dosed animals and animals dosed with a compound that did not cause adverse effects. MGS baseline values in BALB/c showed a median of 1 and a range of 0-2. AA score helped distinguish adverse effects from the effects of handling and procedures. The MGS is a valuable and practical tool to assess mouse welfare during procedures and thus can help identify experimental protocols where a ''pain face'' is observed and there is room for refinement.

PF6

Evidence-based severity assessment in epilepsy models: Identification and validation of parameters in rats

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Abstract
Introduction: To implement the 3R's and improve animal welfare in epilepsy research it is necessary to understand the burden of repeated seizures in rodents. The present study aimed to identify and validate physiological, behavioral and biochemical parameters that provide a basis for evidence-based severity assessment in three epilepsy models (kindling model, electrical post-status epilepticus model, chemical post-status epilepticus model).
Methods: Rats were implanted with electrodes and telemetric devices for wireless EEG and ECG recordings. During epileptogenesis and, in case of the post-status epilepticus models, during the chronic phase different behavioral tests (i.e. saccharin preference test, burrowing paradigm) were performed. Additionally, biochemical parameters (i.e. fecal corticosterone metabolites) were investigated and telemetric ECG and EEG recordings were made.
Results: Rats with a history of a chemically- or electrically-induced status epilepticus showed increased anhedonia-associated behavior in the saccharin preference test whereas kindled rats did not. Moreover, animals of the post-status epilepticus models exhibited reduced burrowing behavior in comparison to controls. In kindled animals this effect was not observed. Additionally, increased fecal corticosterone metabolites were identified in samples from the post-status epilepticus models, but not in those from the kindling paradigm.
Conclusion: The study confirms significant alterations in behavioral and physiological parameters in animals with a history of a status epilepticus while differences in the same parameters were rather mild in the kindling model. This comparison will provide a basis for an evidence-based severity assessment scheme and for an animal welfare based prioritization of epilepsy models.

PF7

Welfare indicator for animals used for scientific purposes: Physiology and welfare considerations

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Abstract
Welfare problems in laboratory animals can be a consequence of an ongoing procedure, modification of the genome, and management mistakes leading to incorrect interpretation of research data. Since a sick or distressed animal could produce unreliable results. A practical protocol for assessing animal welfare in rodents used for scientific purposes were developed for conducting animal experiments according to ethical principles. The protocol provided new indicators, which were obtained from appearance and behavioral changes as signs of the animal welfare state, but also the common signs such as pain, distress and impairment were measured. The expression of appropriate behavior were assessed by analyzing the physical, social and nutritional environment, exploratory behavior, senses (sight, sound, smell, and touch), qualitative behavior assessment, and human-animal relationship (fear, anxiety). To these end, different measurements, raising from 4 welfare principles (good feeding, housing, health, and appropriate behavior) and 12 related welfare criteria, were integrated to new indicators in an objective manner. The integrated measurable indicators were hair coat conditions, feeding (quantified observationally in terms of mechanics and characters), fecal soiling scores, bedding, limping, discharge from the eye and nose, presence of lumps and/or bumps, etc. The collection of these indicators were investigated in rodent behavior and health status and had provided information about the animal well-being allowing us to develop strategies for monitoring pain and distress. In addition, evaluating a new criterion could also determine humane endpoints during procedures as well as playing a key role for effective monitoring of animals health and welfare.

PF8

Severity assessment of CCL4 injection in mice for the induction of liver fibrosis

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Abstract
Introduction: The Directive 2010/63 EU acquires to classify burdens in laboratory animal procedures. Therefore severity assessment in animal models is of major interest. The aim of this study was to assess the severity of the induction of liver fibrosis by intraperitoneal carbontetrachloride (CCL4) injections in mice.
Methods: 24 male C57Bl/6N were treated 3 x per week for 4 weeks with an intraperitoneal injection (50µl) of either 0.6ml/kg CCL4-mixed germ oil solution or germ oil as control. Severity

assessment was performed using clinical scoresheet (Morton DB et al 1985), survival rate, serum analysis and behavioral tests (Mouse Grimace Scale (MGS), OpenField (OF), RotaRod, burrowing and nesting behavior). Humane endpoints were applied when > 20% body weight decrease, enlarged abdomen, vocalisation when grasping, lethargy, hypothermia. Animals were trained before the experiments and baseline measurements were recorded.
Results: The results showed higher severity in week 2 as shown by higher ALT values in the CCl4 application between (CCl4 vs. Control Mean±SD; 3777±4081 vs. 131±173 U/L *p<0.01 2way ANOVA) increase in nesting behavior, less overall burrowing, less moving in OpenField (3845±1525 vs. 6010±1832* cm). This was confirmed by only 50% survival in the CCl4 group. RotaRod showed no significant differences.
Conclusion: The study could demonstrate the general feasibility of behavioral tests for severity assessment. The severity for 50% of the animals in the CCL4 group was severe. In order to refine this model, further examinations are necessary, e.g. the administration of analgesics, oral application of CCL4 or changes in the injection interval and schedule.

PF9

Predicting the severity of rat TNBS-induced colitis using simple parameters

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Abstract
Introduction: TNBS (2,4,6-trinitrobenzene sulfonic acid)-induced colitis is a popular inconsistent rat animal model [1].
Aim: To identify metabolic parameters predictive of the severity of rat TNBS-induced colitis.
Animals and Methods: Protocols were approved by local animal welfare body and national competent authority. On day 0, male Wistar Han rats, 8-12 weeks old, were anesthetized (isoflurane *ad effectum*) and rectally instilled with TNBS (30% ethanolic solution, 20mg/rat, *n*=17) after 24h fasting (sugary solution *ad libitum*). Controls were littermates (n=11). Analgesia was provided by tramadol (20mg/kg, SC, day 1) and acetaminophen (6mg/ml, drinking water). Body weight, food and water intake, fecal excretion, general welfare and grimace scores were daily monitored. On day 7, animals were euthanized by decapitation and the colon macroscopically scored (MaS) to categorize disease severity as Mild (mTNBS, MaS 0-4), Moderate (mdTNBS, MaS 0-8) or Severe (sTNBS, MaS 8-12).
Results: TNBS decreased body weight, food/water intake and fecal excretion. On day 7 mTNBS recovered body weight, while mdTNBS and sTNBS lost weight (6.2% and 4.5%, respectively). By day 5 mTNBS rats normalized food intake and fecal excretion, as did mdTNBS rats on day 6. sTNBS rats never regain food intake or fecal excretion. On day 7 mTNBS rats had lower general welfare and grimace scores than mdTNBS and sTNBS rats.
Conclusion: Food intake and fecal excretion on days 5-6 post induction may predict the severity of rat TNBS-induced colitis.

M. Morato thanks GEDII for funding and Mrs. Céu Pereira for excellent technical assistance.

PF10

Differentiating individual severity levels in group housed mice by voluntary wheel running

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Abstract
Introduction: Voluntary wheel running (VWR) has been proven to be a sensitive method to assess severity in models of Dextran Sulfate Sodium (DSS) colitis and isolation stress in individually housed mice. However, considering refinement (3R's principle), we tested a system for group housed mice with recording of individual VWR using RFID technology.
Methods: The utilized VWR system consisted of six connected, unrestrictedly accessible cages with one wheel each. 21 female C57BL/6J mice underwent subcutaneous transponder implantation under short Isoflurane anesthesia, and, after adaption to running wheels, were subjected to DSS treatment and sampling procedures (SP; collection of blood, feces (1% DSS, n=6-7), SP alone (0% DSS, n=7) or no treatment (control, n=7).
Results: 1% DSS mice showed a significant decrease in VWR and body weight (BW), with a maximum drop on d7. Surprisingly, resulting from SP alone, 0% DSS mice revealed a drop, fully recovering only on d13. Control mice displayed overall stable VWR at baseline level. Neither 0% DSS nor control mice displayed loss of BW. Notably, absolute values of baseline VWR were reduced by approx. 50% compared to singly housed mice.
Conclusion: In this study, VWR was suitable to detect changes in welfare of DSS treated group housed mice. SP affected VWR behavior of 0% DSS mice but not the course of BW. Therefore, VWR can also be used in group housed animals to detect compromised welfare, although, as revealed in reduced VWR compared to single housing, influence of companionship and extension of space should be considered.

PF11

Evaluation of severity assessment strategies during post-operative recovery after laparotomy in mice

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Abstract
Introduction: Telemetric devices are used in laboratory mice to determine physiological and actigraphical parameters. However, the implantation itself is a painful intervention and mice should be monitored very well to minimize pain and suffering, according to the 3R's principle. To monitor post-operative recovery specifically, different physiological and behavioral parameters were analyzed.
Methods: C57BL/6J mice were randomly submitted to transmitter implantation (TI) or sham operation and separated into two analgesic treatment groups, either Metamizol or Carprofen. Post-operatively, they were monitored regularly via clinical scoring, telemetric supervision regarding the temperature, heart rate (HR), heart rate variability (HRV), standard deviation of the NN intervals (SDNN) and activity, the Mouse Grimace Scale (MGS) and the burrowing behavior.
Results: While mice started regaining body weight at the latest on day two post-op, we were able to detect an influence of the transmitter implantation considering the telemetric parameters (HR, HRV, SDNN) for one week. In addition, the MGS showed significant differences in the first three hours in both operation types, whereas the burrowing behavior was affected in TI mice for up to five days.
Conclusions: There are only slight differences between the two administered analgesics. However, sham operated mice seem to be less affected than TI mice. A multimodal analgesia should be reconsidered for TI mice. The MGS is only useful immediately after operation, whereas telemetric data and burrowing behavior show disturbed animal welfare up to one week.

PF12

'One Europe': The challenge of consistency in severity classification

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Abstract
The experience of recent years shows that severity classification is still insufficiently harmonised within the EU. Even among experts, animal procedures are often evaluated very differently. Consistent severity assessment and classification of procedures are essential for the ethical review process, and an indispensable part of planning, refining and evaluating animal experiments. Annual statistics of animals used in scientific research are intended to inform the public about the harms inflicted. Severity classification plays a crucial role in this process, and increased harmonisation should be sought.

As a first step, we are collecting and comparing severity classifications which have been published. The collection is based upon a literature review, integrating severity classifications produced by expert working groups and individual classification schemes. We are constructing an overview of classifications of experimental procedures, initially focusing on severe discomfort, and harmful phenotypes of genetically altered animals. The overview will be made available online at https://norecopa.no/more-resources/severity-classification. The overview will serve as a valuable source for scientists, animal welfare bodies and representatives of the authorities. Severity classifications for specific procedures can be accessed at a glance. The overview will aid identification of areas where more research is needed, and will stimulate discussion so that additional information is submitted. This will hopefully lead to a second step: the refinement of the criteria used for severity classification, on the basis of better informed decisions.

PF13

Consideration on the reuse of immunized female breeders

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Abstract
Reuse of animals in procedures should be ensured when conditions are met based on Article 16 of Directive 2010/63/EU. Limited data are available on the procedures and protocols concerning the assessment of animal reuse and the reproducibility of the respective research data. We share our findings on the reuse of animals through our experience on fetal and neonatal mice tolerization protocols, aiming to reduce anti-drug-antibodies (ADA) in arthritic transgenic models treated with therapeutic monoclonal antibodies (mAb).

We detected the presence of ADA in transgenic offsprings originated from reused mothers. Eight weeks before, in previous experiment, these female breeders had been injected ip with low dose of therapeutic mAb during gestation and early lactation, in order to reduce immunogenicity to pups that would receive mAb treatment. These findings indicate that although offsprings had never been exposed to the mAb, they may have acquired the ADA developed in the reused mothers during the above tolerization protocol.

In conclusion, reuse of mAb-treated breeders should be excluded in therapeutic protocols involving the same mAb. For reduction purposes, we suggest to define the ADA levels in breeders at various time points after mAb administration in order to

evaluate their reuse in subsequent related experimental investigations.

PF14

The burden of intracranial surgery in rat models for neurological disorders

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Abstract
Objectives: The aim of this study is to quantify the actual severity of intracranial surgery with different degree of invasiveness or repeated surgery in a rat Parkinson model and a rat glioblastoma model.
Methods: Adult male rats (Crl:CD and BDIX) experienced these surgical approaches under general anaesthesia: (1) stereotaxic implantation of electrodes for recording and stimulation of neuronal activity, (2) local intracranial injection of 6-hydroxydopamine (6-OHDA) for nigrostriatal loss of dopamine cells (model of Parkinson's disease) or vehicle, followed by stereotaxic implantation of electrodes after four weeks, and (3) intracranial injection of the BT4Ca-rat glioma cell line for intracranial tumor formation, followed by resection of the tumor after one week. The severity assessment was carried out two days before and four days after surgery by clinical scoring, body weight and locomotor activity in the open field.
Results: Stereotaxic vehicle injection, cell injection or electrode implantation had no effect on clinical scores, body weight and locomotor activity, while injection of 6-OHDA resulted in a weight-loss of about 7% and reduced locomotor activity, which lasted until the second operation. Electrode implantation as second operation resulted in a 4% weight reduction with no difference between 6-OHDA and vehicle injected groups. Resection of tumors as second surgery one week after initial glioma cell injection also resulted in a small but significant body weight loss of about 3%, while clinical score and locomotor activity were not affected.
Conclusions: Intracranial neurosurgical interventions of different degree of invasiveness only mildly affect rat's well-being.

PF15

Experimental procedures and their impact on heart rate and activity in rats

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Abstract
Introduction: One fundamental issue in animal experimentation is the best possible severity assessment of experimental procedures. Additionally to clinical scoring, distinct methods, such as grimace scaling or burrowing, have been introduced. In order to determine possible impact of surgery, as well as different handling and experimental procedures, contactless measurement of heart rate (HR) and activity were analyzed by telemetry.
Animals, Materials and Methods: By minimal invasive surgery under general anesthesia a telemetric device was implanted subcutaneously in eight-week-old, male BDIX/UImHanZtm rats. Each rat was randomly subjected to different experimental procedures (e.g., cage change, burrowing, subcutaneous injection, intracranial surgery).
Results: Rats demonstrated transient elevated HRs and changes in activity after surgery, handling and experimental procedures. In order to classify our data, a statistical model on the basis of the post-operative data and baseline data was developed. Entering HR and activity measures from the different procedures into this model revealed that the impact of routine handling (e.g., cage changes), methods for severity assessment (e.g., burrowing) and experimental procedures (e.g., subcutaneous injections) was similar and short-lasting. The effect of subcutaneous transmitter implantation was longer-lasting than intracranial surgery.
Conclusion: The results indicate that the impact of routine and experimental procedures, such as cage change, burrowing, or subcutaneous injection do not substantially differ from each other with respect to HR and activity. However, classification of telemetric data may nevertheless be useful to quantitatively evaluate impact of different procedures during animal experimentation.

PF16

A multifactorial distress analysis of animal models for liver fibrosis

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Abstract
Introduction: In biomedical research, one should choose animal models, which permit high quality of research and cause minimal harm to animals. However, only few publications describe distress caused by experiments or provide methods for comparing the burden of distinct animal models.
Material and Methods: We compared distress of BALB/c mice after bile duct ligation to distress after repetitive carbon tetrachloride injection by analyzing burrowing activity, body weight change and a distress score. Using data of our bile duct ligation model, we determined the accuracy of single as well as combined read out parameters and the optimal cut off in differentiating between distinct distress levels by employing receiver operating characteristic curves, Youden's index and multiple logistic regression. The optimal cut off was then applied to the data set describing distress after carbon tetrachloride injections.

Results: ROC analysis revealed superiority of combining all parameters instead of analyzing single parameters, when assessing distress. We also determined the optimal cut off of our multifactorial distress analysis on mice before and after bile duct ligation. This cut off had a 100% accuracy when applied to a second data set. When applying this cut off to compare distress of mice after bile duct ligation to distress of mice after carbon tetrachloride injections, the analysis revealed significantly higher distress after bile duct ligation.

Conclusion: This multifactorial distress analysis demonstrates that repetitive carbon tetrachloride injection causes less distress than bile duct ligation.